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Abstract: GABAA receptors (GABAA Rs) are ligand-gated Cl(-) channels that mediate most of the fast inhibitory neurotransmission in the central nervous system (CNS). Multiple GABAA R subtypes are assembled from a family of 19 subunit genes, raising the question of the significance of this heterogeneity. In this review, we discuss the evidence that GABAA R subtypes represent distinct receptor populations with a specific spatio-temporal expression pattern in the developing and adult CNS, being endowed with unique functional and pharmacological properties, as well as being differentially regulated at the transcriptional, post-transcriptional and translational levels. GABAA R subtypes are targeted to specific subcellular domains to mediate either synaptic or extrasynaptic transmission, and their action is dynamically regulated by a vast array of molecular mechanisms to adjust the strength of inhibition to the changing needs of neuronal networks. These adaptations involve not only changing the gating or kinetic properties of GABAA Rs, but also modifying the postsynaptic scaffold organised by gephyrin to anchor specific receptor subtypes at postsynaptic sites. The significance of GABAA R heterogeneity is particularly evident during CNS development and adult neurogenesis, with different receptor subtypes fulfilling distinct steps of neuronal differentiation and maturation. Finally, analysis of the specific roles of GABAA R subtypes reveals their involvement in the pathophysiology of major CNS disorders, and opens novel perspectives for therapeutic intervention. In conclusion, GABAA R subtypes represent the substrate of a multifaceted inhibitory neurotransmission system that is dynamically regulated and performs multiple operations, contributing globally to the proper development, function and plasticity of the CNS.

DOI: https://doi.org/10.1111/ejn.12534

Review article (Special Issue)

$GABA_A$ receptors and plasticity of inhibitory neurotransmission in the CNS

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Running title: $GABA_A$ receptors

Keywords: postsynaptic density, gephyrin, phosphorylation, neurogenesis, diazepam, development, GABAergic transmission, plasticity, excitatory-inhibitory balance

Abstract

$GABA_A$ receptors ($GABA_ARs$) are ligand-gated Cl^- channels mediating most of fast inhibitory neurotransmission in the CNS. Multiple $GABA_AR$ subtypes are assembled from a family of 19 subunit genes, raising the question of the significance of this heterogeneity. In this review, we discuss the evidence that $GABA_AR$ subtypes represent distinct receptor populations with a specific spatio-temporal expression pattern in developing and adult CNS, being endowed with unique functional and pharmacological properties, as well as being differentially regulated at the (post-) transcriptional and translational levels. $GABA_AR$ subtypes are targeted to specific subcellular domains to mediate either synaptic or extrasynaptic transmission, and their action is dynamically regulated by a vast array of molecular mechanisms to adjust the strength of inhibition to the changing needs of neuronal networks. These adaptations take place not only by changing the gating or kinetic properties of $GABA_ARs$, but also by modifying the postsynaptic scaffold organized by gephyrin to anchor specific receptor subtypes at postsynaptic sites. The significance of $GABA_AR$ heterogeneity is particularly evident during CNS development and adult neurogenesis, with different receptor subtypes fulfilling distinct steps of neuronal differentiation and maturation. Finally, the analysis of the specific role of $GABA_AR$ subtypes reveals their implication in the pathophysiology of major CNS disorders and opens novel perspectives for therapeutic intervention. In conclusion, $GABA_AR$ subtypes represent the substrate of a multi-facetted inhibitory neurotransmission system, which is dynamically regulated and performs multiple operations contributing globally to proper development, function and plasticity of the CNS.

Introduction

$GABA_A$ receptors ($GABA_ARs$) belong to the family of Cys-loop ligand-gated ion channels, along with nicotinic acetylcholine receptors, glycine receptors (GlyR), and serotonin type 3 receptors ($5\text{-}HT_3$), which form pentameric channels carrying two extracellular ligand binding sites (Olsen & Sieghart, 2008). Being permeable for Cl^- ions, $GABA_ARs$ mediate most of the inhibitory action of GABA in the CNS. By virtue of their ubiquitous expression in neurons (and possibly glial cells; (Passlick *et al.*, 2013)), $GABA_AR$ contribute to all CNS functions, including sensory and motor processing, central autonomic control, sleep-wakefulness, emotions, and cognition. Clinically, the main relevance of $GABA_AR$ relates to their exclusive targeting by benzodiazepines (and other ligands with high affinity for the benzodiazepine binding site), used for their anxiolytic, sedative, anticonvulsant, and muscle relaxant properties. Benzodiazepine site ligands act as allosteric modulators, and, due to their extraordinary selectivity, produce no other direct action in the CNS. $GABA_ARs$ are also the target of general anesthetics (Rudolph & Antkowiak, 2004), ethanol, and endogenous modulators, notably endozepines (Christian *et al.*, 2013) and neurosteroids (Hosie *et al.*, 2006). These ligands, in large part derived from glial cells, are considered to be crucial regulators of neuronal function and excitability under both physiological and pathological conditions and represent promising novel targets for specific neurological and psychiatric indications (reviewed in (Belelli & Lambert, 2005; Carver & Reddy, 2013)).

In the early '90s, the identification of 19 genes encoding $GABA_AR$ subunits ($\alpha1$-$\alpha6$, $\beta1$-$\beta3$, $\gamma1$-$\gamma3$, δ, ε, π, τ, $\rho1$-$r3$) in mammals, coupled with the demonstration that recombinant receptors assembled with an α, a β and the $\gamma2$ subunit variant were functionally and pharmacologically similar to native $GABA_ARs$, raised the question of the significance of $GABA_AR$ heterogeneity. This question is still largely unresolved, but the evidence available suggests that $GABA_AR$ subtypes, differing in subunit composition, represent distinct functional entities, imparted with specific functions and pharmacological profile, and with a unique spatio-temporal mRNA and protein expression pattern. In this review, we will discuss this evidence, based on four fundamental observations made since the existence of $GABA_AR$ subtypes was established:

A first major thrust in understanding the function of $GABA_AR$ subtypes and the relevance of their molecular heterogeneity has been the recognition that they mediate two fundamentally distinct forms of inhibitory transmission, which depend on their localization, either postsynaptic (mediating fast, high amplitude phasic currents upon quantal presynaptic GABA release) or extrasynaptic (mediating low amplitude but persistent (tonic) currents activated by ambient GABA) (reviewed in (Farrant & Nusser, 2005; Belelli *et al.*, 2009; Brickley & Mody, 2012)) (see Box 3). The significance of tonic inhibition, in particular for the control of neuronal excitability and plasticity, is now gaining widespread recognition. Importantly, these two major populations of $GABA_AR$ are molecularly

distinct, with postsynaptic receptors containing mainly the α1, α2, α3 subunit, along with β subunit variants and the γ2 subunit, and extrasynaptic receptors containing α4, α5, α6 subunit, often along with δ (instead of γ2). This observation implies that mechanisms of subcellular targeting of $GABA_AR$ subtypes are subunit-specific, and can vary between CNS regions and developmental stages. The crucial role played by the postsynaptic scaffold organized by gephyrin for post-synaptic targeting and confinement of some $GABA_ARs$ in GABAergic postsynaptic densities (PSDs) is now widely recognized, albeit poorly understood (Fritschy *et al.*, 2012; Tretter *et al.*, 2012). Further, there is ample evidence for modulation of both postsynaptic and extrasynaptic $GABA_ARs$ by multiple posttranslational mechanisms regulating single channel functional properties, trafficking (exo- and endocytosis, degradation), cell surface mobility and synaptic confinement. Thereby, these mechanisms have a major impact on the strength of GABAergic transmission in response to changes in network function, and they represent a major facet of GABAergic synapse plasticity (Hines *et al.*, 2011; Luscher *et al.*, 2011a; Connelly *et al.*, 2013a).

A second major advance was the demonstration, using gene targeting techniques, that the spectrum of diazepam's actions is elicited by distinct $GABA_AR$ subtypes, distinguished by their α subunit variant (Rudolph & Möhler, 2004). The logical consequence of these findings is that these distinct $GABA_ARs$ are localized in different neuronal circuits, even when they are co-expressed within neurons or within specific brain areas (see Box 2). An important concept emerging along this line is that GABAergic interneurons are likewise specialized to control the activity of principal cells in a circuit-specific manner (Klausberger & Somogyi, 2008). This highly sophisticated organization raises the possibility that multiple forms of inhibitory neurotransmission, engaging specific interneurons and $GABA_AR$ subtypes, operate in parallel in neuronal circuits involved in concurrent tasks. The possibility to probe interneuron function *in vivo* using optogenetic tools has substantially advanced our understanding of their role and functional specialization (Sohal *et al.*, 2009; Pfeffer *et al.*, 2013; Zhu *et al.*, 2013).

A third fundamental insight into $GABA_AR$ function is their dependence on ionic mechanisms, involving both Cl^- and HCO_3^- ions (Blaesse *et al.*, 2009). Therefore, the effects of $GABA_ARs$ on the resting membrane potential, independently of their subunit composition, are determined by the action of KCl co-transporters and carbonic anhydrases (see Box 4). It has been recognized early that the expression of KCC2, the main Cl^- extrusion transporter in mature neurons (Kaila, 1994), is developmentally regulated, giving rise to the concept of a functional "switch" from depolarizing to hyperpolarizing $GABA_AR$ actions during ontogeny (Ben-Ari, 2002), with wide ranging consequences to our understanding of GABA function, as well as clinical use of $GABA_AR$-modulating drugs in infants (Pavlov *et al.*, 2013).

Fourth, it is now well established that $GABA_AR$-mediated transmission regulates multiple steps of neuronal development and maturation during ontogenesis and adult neurogenesis, including control of

stem/precursor cell proliferation, cell fate decision, migration of precursor cells, survival of immature neurons, dendritic growth, and synaptogenesis (reviewed in (Platel *et al.*, 2007; Dieni *et al.*, 2013)). Accordingly, it is being increasingly recognized that perturbations of $GABA_AR$ function during ontogeny or after a lesion, notably during critical periods of plasticity, can have long lasting effects on CNS circuit structure and function, potentially contributing to the pathophysiology of neurological and psychiatric disorders, including epilepsies, chronic pain, neurodevelopmental disorders, mood disorders, and schizophrenia (Bavelier *et al.*, 2010; Lewis, 2012).

Here, we will review the relevance of $GABA_AR$ heterogeneity for the regulation of GABAergic neurotransmission and GABAergic synaptic plasticity, and its potential impact for the pathophysiology of major neurological and psychiatric diseases, notably disorders linked to abnormal GABAergic function during brain development. We will highlight recent progress and discuss major roadblocks on the way to better understand the diversity of $GABA_AR$ regulation in health and disease. To do so, we will briefly introduce the molecular heterogeneity of GABAergic synapses, prior to discussing mechanisms regulating $GABA_ARs$ in the context of GABAergic synaptic plasticity. Next, we will highlight the relevance of $GABA_AR$ subtypes for regulating neuronal development, and finally, for the pathophysiology of neurological and psychiatric diseases linked to abnormal GABAergic transmission.

Composition and localization of major $GABA_A$ receptor subtypes

The subunit composition of major $GABA_AR$ subtypes is well established on a regional level (immunoprecipitation using extracts from whole brain or a specific brain region), and there is general agreement that the most likely subunit stoichiometry is $2\alpha/2\beta/\gamma$ (the latter being sometimes substituted by δ or ε) (Boileau *et al.*, 2005; Olsen & Sieghart, 2008; Patel *et al.*, 2013). The existence of receptors containing α/β subunits only, as well as other stoichiometries (e.g. $2\alpha/\beta/2\gamma$, $2\alpha/\beta/2\varepsilon$) is probable (Jones & Henderson, 2013). Furthermore, the rules governing the formation of pentameric complexes are by far not fully elucidated. Functional $GABA_ARs$, with the pharmacological profile of native receptors, are formed by pentameric assembly of $2\alpha/2\beta/\gamma2$, whereby the α and β subunits can be either identical or different. The $\gamma2$ subunit can be substituted by $\gamma1$ or $\gamma3$ (present at low abundance and/or with a restricted expression pattern) or by δ, and possibly ε subunits. There is consensus, therefore, that at least 3 dozen distinct $GABA_AR$ subtypes exist in CNS neurons (Olsen & Sieghart, 2008). *In vitro* expression of β/γ subunits only, or targeted deletion of an α subunit gene *in vivo*, prevent assembly and/or surface targeting of a functional $GABA_AR$ complex, thereby providing the opportunity to remove specific $GABA_AR$ subtypes by inactivating a single α subunit gene (see Box 1).

The most detailed information on the regional distribution of 18 $GABA_AR$ subunit mRNAs in mouse brain, determined by non-radioactive *in situ* hybridization with cellular resolution, is available in the

Allen Brain Atlas (http://mouse.brain-map.org). These data confirm original studies about the distribution of abundant subunits (α1-α6, β1-3, γ2) (Laurie *et al.*, 1992; Wisden *et al.*, 1992) and provide detailed information about subunits expressed either at low abundance in numerous regions throughout the neuraxis, such as γ3, or at high abundance in specific regions, such as ε in the amygdala, basal forebrain, locus coeruleus (and other noradrenergic cell groups). According to the Allen Brain Atlas, the ρ1-ρ2 mRNAs (corresponding to $GABA_C$ receptors) are restricted to the superficial layers of the superior colliculus and the π subunit mRNA is undetectable in adult mouse brain.

Immunohistochemically, the distribution of ten subunits (α1-α6, β2-β3, γ2, δ) has been analyzed in detail, on the regional and sometimes cellular level, and validated by targeted gene deletion (Fritschy & Mohler, 1995; Nusser *et al.*, 1999; Peng *et al.*, 2002; Chandra *et al.*, 2006; Hörtnagl *et al.*, 2013). There is also information available for the β1 and γ1 subunit, which confirms *in situ* hybridization data. According to these studies, it is well apparent that the six α subunit variants largely correspond to distinct $GABA_AR$ subtypes – notwithstanding the possibility to find two different α subunits in a substantial fraction of $GABA_ARs$ (Balic *et al.*, 2009) – each with a specific distribution pattern that overlaps only partially with other α subunits (Figure 1). The same holds true for the β subunit variants, with β2 and β3 overlapping to a large extent with the α1 and α2 subunits, respectively (whereas β1 is expressed at lower abundance in numerous brain regions). The γ2 subunit, in line with its association with the vast majority of $GABA_AR$ subtypes, is ubiquitously expressed; in contrast, the γ1 subunit appears to have a highly restricted distribution, being most abundant in hypothalamus, amygdala, and parts of basal ganglia, as well as the inferior olivary nucleus. Finally, the δ subunit, which forms $GABA_AR$ located extrasynaptically (see Box 3), largely overlaps with the α4 subunit in the forebrain and with α6 in the cerebellum.

So far, however, there are only few CNS regions in which the $GABA_AR$ subunit repertoire and their cellular/subcellular distribution have been analyzed in some detail by immunohistochemistry. These include the hippocampal formation (notably CA1 subfield, which exhibits a remarkable heterogeneity with expression of at least 11 subunits), neocortex, olfactory bulb, parts of the thalamus (notably, ventrobasal complex, lateral geniculate nucleus, and reticular nucleus), cerebellum, and spinal cord dorsal horn.

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* Box 1 approximately here *

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On the subcellular level, a distinction between postsynaptic and extra-synaptic $GABA_ARs$ can be made, based on the appearance of staining in weakly fixed tissue (see Box 1). Postsynaptic receptors

form brightly stained clusters, which co-localize with postsynaptic markers, such as gephyrin and neuroligin2, and are apposed to VGAT-positive presynaptic terminals (Fig. 2A). The reminder of the staining, besides these clusters, represents receptors dispersed at the cell surface, as well as the metabolic pool of receptors localized in the cytoplasm. Extrasynaptic receptors fail to form clusters, and the staining is of uniform intensity, with a "powdery" appearance in the neuropil, suggesting a widespread distribution on dendritic branches (Fig. 2B). Accordingly, these structures exhibit no obvious relationship with the distribution of either gephyrin or GABAergic axon terminals (Fig. 2B). As discussed below, presynaptic receptors localized in axons and axon terminals represent a distinct subset of extrasynaptic receptors, with specialized functions.

Despite scant morphological evidence from immunohistochemical studies, there is strong functional support for the existence of $GABA_A$Rs located on axons and presynaptic terminals (Grasshoff *et al.*, 2007; Trigo *et al.*, 2008; Long *et al.*, 2009; Witschi *et al.*, 2011). A prominent exception is a population of $GABA_A$Rs, readily detected immunohistochemically for the presence of α2 subunit, located on the axon initial segment of cortical neurons, typically clustered in rows of synapses innervated by axo-axonic interneurons (Nusser *et al.*, 1996; Fritschy *et al.*, 1998; Panzanelli *et al.*, 2011). Functionally, these $GABA_A$Rs correspond to postsynaptic receptors anchored by gephyrin, and their activation contributes to the genesis of γ-oscillations (Tukker *et al.*, 2007). In contrast to axo-axonic synapses, most presynaptic $GABA_A$Rs located on distal axons and terminals are hardly detectable by immunohistochemistry. They nevertheless play a key role in the control of axon potential transmission, neuronal synchronization, regulation of transmitter release, and mediation of presynaptic afferent depolarization (Trigo *et al.*, 2008; Long *et al.*, 2009; Ruiz *et al.*, 2010; Wakita *et al.*, 2013). While their subunit composition is unknown, it might be inferred from the repertoire of subunit mRNAs expressed by the cell of origin of these axons. We have characterized recently a population of $GABA_A$R on primary afferent terminals in the spinal cord, containing α2 and α3 subunits, that are crucially involved in the anti-hyperalgesic action of diazepam (Witschi *et al.*, 2011; Paul *et al.*, 2012).

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* Box 2 approximately here *
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Molecular organization of GABAergic synapses

The subcellular localization of $GABA_A$Rs is intimately linked to the molecular organization of GABAergic synapses, with specific proteins of the PSD contributing to trafficking and anchoring $GABA_A$Rs in a subtype-specific manner. It has been recognized early that GlyRs, which are homologous to $GABA_A$Rs, are localized at postsynaptic sites (Triller *et al.*, 1985), owing to their high

affinity binding to the scaffolding protein gephyrin (Pfeiffer *et al.*, 1982; Kirsch *et al.*, 1993), which interacts with the cytoskeleton. The demonstration that the majority of postsynaptic $GABA_A$Rs also are clustered with gephyrin took longer to be obtained (Sassoè-Pognetto *et al.*, 1995; Sassoè-Pognetto *et al.*, 2000), in part because gephyrin was considered to be present only at glycinergic synapses, and in part because $GABA_A$Rs do not bind gephyrin with high affinity. Based on the evidence available to date, the α1, α2, and α3 subunits, as well as possibly β2 and β3, can interact directly with gephyrin via motives located in their main intracellular loop (Tretter *et al.*, 2008; Mukherjee *et al.*, 2011; Tretter *et al.*, 2011; Kowalczyk *et al.*, 2013), and, therefore, can be clustered postsynaptically. Conversely, $GABA_A$R containing the α4, α5, or α6 subunit are localized predominantly extrasynaptically (Nusser *et al.*, 1998; Chandra *et al.*, 2006; Panzanelli *et al.*, 2011). The α4 and α6 subunits – as well as α1 in specific interneurons (Mann & Mody, 2010; Milenkovic *et al.*, 2013) – are frequently associated with the δ subunit, which substitutes for γ2 (see Box 3). However, extra- versus postsynaptic targeting of $GABA_A$Rs appear to depend on motifs present in α subunit variants, as seen by comparing recombinant α2- and α6-$GABA_A$Rs containing either γ2 or δ subunit (Wu *et al.*, 2012).

The finding that targeted deletion of the γ2 subunit abolishes postsynaptic clustering of both, $GABA_A$Rs and gephyrin, highlighted the interdependence between receptors (rather than individual subunits) and the gephyrin scaffold for proper localization at the cell surface (Essrich *et al.*, 1998; Schweizer *et al.*, 2003). Likewise, targeted deletion of *Gphn* (encoding gephyrin) confirmed its key role for postsynaptic clustering of GlyRs and $GABA_A$Rs (Feng *et al.*, 1998). These observations triggered intense research into elucidating the functions of gephyrin, and identifying proteins regulating $GABA_A$R trafficking and GABAergic synapse formation (reviewed in (Luscher *et al.*, 2011a; Fritschy *et al.*, 2012; Tretter *et al.*, 2012)). These molecules include, in particular, collybistin, a Rho-GEF identified as direct binding partner of gephyrin, mediating its translocation to the cell surface (Kins *et al.*, 2000), neuroligin2 (NL2), which interacts transsynaptically with neurexin isoforms (Varoqueaux *et al.*, 2004; Kang *et al.*, 2008) (see Fig. 3), as well as an array of proteins regulating $GABA_A$R biogenesis, cell surface trafficking, endocytosis and degradation (Jacob *et al.*, 2008; Luscher *et al.*, 2011a; Vithlani *et al.*, 2011).

Gephyrin: This phylogenetically ancient, highly conserved multi-functional molecule, is responsible throughout the living kingdom for Moco biosynthesis (Stallmeyer *et al.*, 1999). Moco activates Molybdenum enzymes, whose functions are essential for survival (Schwarz *et al.*, 2009). Gephyrin comprises two main catalytic domains (G and E), which in bacteria are encoded by two distinct genes (MogA and MoeA); in plants, the domains are fused, and in vertebrates, they are interconnected by a flexible linker (or central domain), which carries numerous sites for posttranslational modifications and interaction with partner proteins (reviewed in (Fritschy *et al.*, 2008)). The domain organization of mammalian gephyrin not only ensures optimal Moco synthesis, but also allowed the emergence of novel, neuron-specific functions for the regulation of GlyR and $GABA_A$R clustering (Belaidi &

Schwarz, 2013). Whereas the structure of gephyrin is not fully resolved, current evidence suggests formation of trimers (Sander *et al.*, 2013), which organize themselves in a highly ordered supramolecular complex anchoring $GABA_A$Rs in the plasma membrane and interacting with effector proteins (including NL2 and collybistin) and the cytoskeleton (Fig. 3) (reviewed in (Tyagarajan & Fritschy, 2014)).

Postsynaptic clustering of gephyrin by auto-aggregation at GABAergic (and glycinergic synapses) represents a process fundamentally different from scaffold formation in the PSD of glutamatergic synapses, where PSD-95 and its homologues assemble a modular scaffold and interact with their partners by means of the PDZ interaction domain (Kennedy, 2000; Sheng & Sala, 2001). In GABAergic PSDs, most proteins lack a PDZ domain; therefore, there must be molecular mechanisms specifically enabling gephyrin scaffold formation at postsynaptic sites. Identifying these mechanisms is complicated by the fact that biochemical purification of GABAergic PSDs has not been achieved so far, whereas glutamatergic PSDs are highly enriched in synaptosomal fractions (Husi & Grant, 2001). Therefore, there are only limited proteomic data of inhibitory PSDs yet (Heller *et al.*, 2012), and the possibility exists that major players involved in scaffold formation and regulation at GABAergic synapses are not yet identified.

A major insight from recent studies was the finding that posttranslational modifications of gephyrin, mainly via phosphorylation, regulate its clustering properties, and thereby, the structural and functional properties of GABAergic synapses (Tyagarajan & Fritschy, 2014). This regulation is coupled to complex interactions with collybistin and NL2 (see below), contributing to GABAergic synapse formation and their dynamic regulation by activity-dependent mechanisms. Therefore, gephyrin emerges as a key element of a signaling hub regulating GABAergic function and plasticity in health and disease.

It is of note that postsynaptic gephyrin clustering is not an intrinsic property of this protein, but it depends strictly on the presence of $GABA_A$R subtypes with which gephyrin (and possibly additional proteins) can interact. Therefore, the loss of postsynaptic currents in α1- and α2-KO mice also leads to disruption of gephyrin postsynaptic clustering (Fritschy *et al.*, 2006; Kralic *et al.*, 2006; Pallotto *et al.*, 2012). Despite these crucial bidirectional interactions, gephyrin-independent $GABA_A$R clustering at postsynaptic sites has been observed, both *in vitro* and *in vivo* (Kneussel et al., 2001; Levi et al., 2002; Panzanelli et al., 2011), pointing to alternative pathways regulating their synaptic localization. One of these mechanisms might be afforded by the dystrophin-glycoprotein complex (DGC), which is present selectively in a subset of GABAergic synapses in cortical neurons and cerebellar Purkinje cells (reviewed in (Fritschy *et al.*, 2012)). Indeed, we have shown in α2-KO mice that postsynaptic clusters of α1-$GABA_A$Rs and NL2 remain selectively associated with the DGC, but not with gephyrin, in perisomatic synapses of CA1 pyramidal cells (Panzanelli *et al.*, 2011). A possible molecular link holding these proteins together is provided by synaptic scaffolding molecule (S-SCAM), which

interacts with both, dystrophin and NL2 (Sumita *et al.*, 2007). Interestingly, the cell adhesion molecule IgSF9b, interacting with S-SCAM and NL2, has recently been identified to promote formation of GABAergic synapses, in particular in cortical interneurons, where it is most strongly expressed (Woo *et al.*, 2013).

A recent study using super-resolution microscopy to analyze the molecular organization of inhibitory PSDs provided a first quantification of the number of gephyrin molecules present per synapse, and demonstrated that the number of both GlyRs and $GABA_A$Rs at the synapse depend on gephyrin abundance (Specht *et al.*, 2013). A possible difference in the molecular organization of these synapses emerged from the observation that silencing neuronal cultures with tetrodotoxin for 48 h affected the synaptic enrichment of $GABA_A$Rs, but not GlyRs.

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* Box 3 approximately here *

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Collybistin is a neuron-specific guanine nucleotide exchange factor that activates the small Rho GTPases CDC-42 and TC-10 (Mayer *et al.*, 2013), and binds gephyrin at an identified site. Loss of $GABA_A$R and gephyrin clustering occurs in a cell type-specific manner in collybistin-KO mice, unraveling the essential function of this protein at GABAergic synapses (Papadopoulos *et al.*, 2007; Papadopoulos *et al.*, 2008). The effect can be reproduced in cultured neurons upon over-expression of a collybistin isoform unable to interact with membrane phospholipids (via its PH domain) (Reddy-Alla *et al.*, 2010; Tyagarajan *et al.*, 2011a). In these experiments, expression of constitutively active CDC-42 restored gephyrin clustering, indicating that it probably operates down-stream of collybistin (Tyagarajan *et al.*, 2011a). However, elucidating the function(s) of collybistin and its effectors is rendered complicated by the existence of collybistin splice variants, carrying or lacking an N-terminal SH3 domain ($collybistin_{SH3+}$ and $collybistin_{SH3–}$, respectively), because $collybistin_{SH3+}$ has been reported to be an inactive form that needs to be activated in order to contribute to gephyrin and $GABA_A$R clustering (Poulopoulos *et al.*, 2009). The nature of this activation presumably involves a conformational change that enhances binding of the PH domain to membrane phospholipids (Figure 3). It has been proposed, for example, that binding of $GABA_A$R α2 or α3 subunit to collybistin, in conjunction with gephyrin, facilitates gephyrin cluster formation (Saiepour *et al.*, 2010). Likewise, binding of NL2 to the SH3 domain was suggested to activate collybistin and thereby enable gephyrin clustering (Poulopoulos *et al.*, 2009). However, targeted deletion of NLGN2 does not abolish gephyrin clustering and collybistin over-expression in neurons strongly stimulates gephyrin clustering independently of the presence/absence of the SH3 domain (Chiou *et al.*, 2011; Tyagarajan *et al.*, 2011a), suggesting alternative mechanisms. Among these, the small GTPase TC-10 was shown recently to activate collybistin upon interaction with the PH domain, thereby enhancing gephyrin clustering in cultured neurons (Mayer *et al.*, 2013).

Neuroligin 2: There are four neuroligin isoforms encoded by distinct genes (NLGN1-NLGN4) (Bolliger *et al.*, 2001). These proteins, anchored postsynaptically by means of a single transmembrane domain, interact with presynaptic neurexins. This interaction is strongly synaptogenic, even in non-neuronal cells, upon over-expression *in vitro* (Scheiffele *et al.*, 2000; Graf *et al.*, 2004; Chih *et al.*, 2005). NL2 is selectively located at GABAergic synapses (Varoqueaux *et al.*, 2004) and NLGN4 at glycinergic synapses ((Hoon *et al.*, 2011); but see (Soto *et al.*, 2011)), whereas NLGN1 is selective for glutamatergic synapses (Song *et al.*, 1999) and NLGN3 is found in both glutamatergic and GABAergic (Budreck & Scheiffele, 2007). NLGNs have raised considerable attention because they are associated with autism-spectrum disorders and other forms of mental retardation (reviewed in (Südhof, 2008)), as well as schizophrenia (Sun *et al.*, 2011). Their specific *in vivo* role is not yet fully elucidated, in part because of functional redundancy with other synaptogenic molecules (Varoqueaux *et al.*, 2006), and because their functions (and localization) are modulated by complex interaction with neurexin isoforms (Futai *et al.*, 2013), post-translational modifications (Peixoto *et al.*, 2012; Suzuki *et al.*, 2012; Giannone *et al.*, 2013), and homo- and heterodimerization (Poulopoulos *et al.*, 2012; Shipman & Nicoll, 2012). In particular, it is not known whether NLGNs interact directly with $GABA_A$Rs. Nevertheless, NL2-KO exhibit specific reduction of perisomatic GABAergic synapses in principal neurons of the hippocampal formation, associated with reduction of inhibitory currents and increased network excitability (Jedlicka *et al.*, 2011).

It should be noted that while NL2 is generally considered to be upstream in the chain of molecular events leading to formation of GABAergic synapses (Dong *et al.*, 2007; Poulopoulos *et al.*, 2009), *in vitro* evidence indicates that overexpression of $GABA_A$R alone in non-neuronal cells (lacking collybistin and presumably gephyrin) is sufficient to generate functional contacts, generating inhibitory postsynaptic currents (Fuchs *et al.*, 2013).

Functional regulation of $GABA_A$ receptor subtypes: significance for GABAergic synapse plasticity

$GABA_A$Rs are regulated by ubiquitous transcriptional and post-translational processes, as well as by multiple protein-protein interactions. As discussed above with regards to their interactions with gephyrin, there is considerable subtype specificity in the regulation of $GABA_A$Rs, which allows neurons expressing several receptor subtypes to make fine, synapse-specific adjustments in response to a large array of extrinsic and intrinsic signals. Here, we will discuss the regulation of $GABA_A$R subtypes in mature neurons, focusing on four major aspects:

Transcriptional control of $GABA_A$ receptor subunit expression: The gene structure and chromosomal localization of human (and rodent) $GABA_A$Rs are well established (Simon *et al.*, 2004), and their promoter sequences and binding sites for transcription factors and regulatory elements subject to

intense scrutiny (reviewed in (Steiger & Russek, 2004)). Still, little is known how the subunit repertoire of any given neuron is determined during development, although models explaining the coordinated expression of subunits located in gene clusters (e.g., β2-α1-γ2-α6) have been proposed (Uusi-Oukari *et al.*, 2000; Joyce, 2007). A remarkable variability in the abundance of mRNAs encoding the 19 $GABA_AR$ subunits has been uncovered in both mice and human, with considerable regional specificity, and being under the control of multiple gene regulatory mechanisms (Mulligan *et al.*, 2012). In addition, there is strong evidence for transcriptional regulation of $GABA_AR$ subunits by neurosteroids, as well as in a number of pathological conditions, including epilepsy, ethanol intoxication, Alzheimer's disease, and schizophrenia (reviewed in (Steiger & Russek, 2004; Grabenstatter *et al.*, 2012)). A recent genetic study identified a chromosomal duplication in a locus encoding four $GABA_AR$ subunits (4p12; α2, α4, β1, γ1) associated with neurodevelopmental disorders (Polan *et al.*, 2013). In contrast, it is not well established whether the compensatory increase in subunit expression observed in some $GABA_AR$ subunit KO mice (see Box 1) reflects transcriptional control or is due to changes in mRNA stability and/or by post-translational mechanisms (Peng *et al.*, 2002; Kralic *et al.*, 2006; Ogris *et al.*, 2006). The issue is of relevance, because these compensatory changes contribute to maintain homeostatic balance between excitation and inhibition in the mutant mice. Transcriptional control of $GABA_AR$ subunit expression would probably involve activity-dependent mechanisms targeting specific transcription factors (or possible non-coding RNAs), and would need to deal with the regulation of local translation in dendrites (Cajigas *et al.*, 2012).

Posttranslational modifications of $GABA_A$ receptors: Membrane-anchoring of $GABA_ARs$ is regulated by palmitoylation of the γ2 subunit and this mechanism contributes to normal formation and function of GABAergic synapses (Fang *et al.*, 2006). In addition, it is well established that multiple phosphorylation mechanisms, targeting various $GABA_AR$ subunits, play a key role in modulating the efficacy of GABAergic transmission, either by changing single-channel gating or kinetic properties, or by regulating stability, cell-surface delivery, or internalization of $GABA_ARs$ (reviewed in (Jacob *et al.*, 2008; Houston *et al.*, 2009; Luscher *et al.*, 2011a; Vithlani *et al.*, 2011)). Combined with recent reports that gephyrin phosphorylation at residues S268 and S270 by GSK3β and ERK, respectively, is a negative regulator of GABAergic transmission (Tyagarajan *et al.*, 2011b; Rui *et al.*, 2013; Tyagarajan *et al.*, 2013), these data indicate that multiple signaling pathways can dynamically modulate neuronal excitability by activating protein kinases or phosphatases, as well as their downstream effectors. As phosphorylation events on $GABA_ARs$ are subunit-specific, differential effects can be expected for various $GABA_AR$ subtypes, even within the same neuron. Furthermore, considering the tight functional coupling between the gephyrin scaffold and postsynaptic $GABA_ARs$, the question arises whether phosphorylation of gephyrin and $GABA_ARs$ is coordinated. As gephyrin carries multiple consensus sites for phosphorylation (and other post-translational modifications, such as acetylation and SUMOylation) (Tyagarajan & Fritschy, 2014), the response to this question awaits

their further characterization. In addition, it is conceivable that the gephyrin scaffold serves to anchor protein kinases (and phosphatases) acting on $GABA_A$Rs and therefore regulates the efficacy of receptor posttranslational modifications.

A major advance in our understanding of the *in vivo* significance of $GABA_A$R phosphorylation is provided by the generation of knock-in mice carrying point-mutations that abolish phosphorylation of residues known to be targeted by protein kinases *in vitro*. However, these studies have unraveled unexpectedly strong effects of the mutations, with γ2(Y365/367F) mutation being embryonically lethal, and inducing in heterozygous mice sex-specific increased in tonic inhibition (α4/δ-GABAARs) to compensate for reduced neurosteroid sensitivity in the thalamus (Jurd & Moss, 2010; Nani *et al.*, 2013).

Regulation of $GABA_A$ receptor trafficking and cell-surface diffusion: Membrane insertion (and internalization) of $GABA_A$Rs occurs at extrasynaptic sites, followed by lateral diffusion and reversible trapping in the postsynaptic membrane (Bogdanov *et al.*, 2006), suggesting dynamic regulation of phasic inhibition from a reserve pool of extrasynaptic receptors (Thomas *et al.*, 2005). Furthermore, preventing docking of $GABA_A$Rs at endocytotic zones in the plasma membrane – by interfering with a binding motif located in the intracellular loop of the β3 subunit – blocked $GABA_A$R internalization, as well as down-regulation following oxygen-glucose deprivation, a model of ischemia *in vitro* (Smith *et al.*, 2012). Such observations underscore that regulated diffusion of $GABA_A$Rs in the plasma membrane is of prime relevance under physiological and pathophysiological conditions.

Single-particle tracking studies also revealed that $GABA_A$R mobility at the cell surface and at postsynaptic sites is tightly regulated by activity-dependent mechanisms and differential interactions with gephyrin (Bannai *et al.*, 2009; Shrivastava *et al.*, 2011; Niwa *et al.*, 2012). Thus, postsynaptic and extrasynaptic $GABA_A$Rs have similar diffusion rates in the plasma membrane, but the former remain confined longer in GABAergic postsynaptic sites and their trapping depended of presence of the gephyrin scaffold (Mukherjee *et al.*, 2011; Renner *et al.*, 2012). Increasing synaptic activity, leading to Ca^{2+} influx, reduced the amplitude of mIPSCs, due to dispersion of $GABA_A$R to extrasynaptic sites by a mechanism involving the protein phosphatase calcineurin (Bannai *et al.*, 2009). Recent evidence indicates, however, that this activity-dependent Ca^{2+} influx also affects the gephyrin scaffold, although dispersion of $GABA_A$Rs and gephyrin occur with different time scales, partially independently of each other (Niwa *et al.*, 2012). Taken together with previous evidence (Muir *et al.*, 2010), these results indicate that cross-talk between excitatory and inhibitory transmission occurs via the activation of Ca^{2+}-dependent signaling events that impinge on both $GABA_A$Rs and the postsynaptic scaffold. Much remains to be determined how these phenomena observed *in vitro* are mediated *in vivo*, and whether they selectively affect specific $GABA_A$R subtypes in cortical principal cells or cause global effects across various types of synapses.

Regulation of $GABA_AR$-mediated transmission by neurotrophins and metabolic factors: Neurotrophins, such as BDNF, have major effects on excitatory synaptic plasticity, mediated by multiple signaling pathways downstream of TrkB. It is, therefore, no surprise that BDNF also regulates the strength of $GABA_AR$-mediated transmission, by acting on both GABAergic synapse formation (Chen *et al.*, 2011) and plasticity: BDNF produces bi-phasic effects on $GABA_AR$-mediated transmission, reflecting its action on cell surface expression (Brünig *et al.*, 2001; Jovanovic *et al.*, 2004). In a recent study, the possibility has been raised that BDNF acutely causes internalization of α1-$GABA_ARs$ in the amygdala by causing rapid gephyrin degradation (Mou *et al.*, 2013). However, a long-lasting enhancement of $GABA_AR$-mediated transmission in the hippocampus, due to enhanced cell surface expression, has been shown to arise from phosphorylation of the tyrosine residues Y657 and Y367 in the γ2 subunit (Vithlani *et al.*, 2013); the significance of this modulation, as tested in γ2(Y365/367F) knock-in mice, is anti-depressant behavioral phenotype and increased hippocampal neurogenesis, raising the possibility that $GABA_AR$ phosphorylation regulates the anti-depressant action of BDNF.

We have recently uncovered a novel mode of $GABA_AR$ regulation, activated by reactive oxygen species (ROS) and, therefore, by cellular metabolism (Accardi *et al.*, 2014). In this study, blocking the mitochondrial respiratory chain, or elevating intracellular ROS in cerebellar interneurons, caused a gradual increase in the frequency of mIPSCs, due to the appearance of additional low amplitude currents with slow decay kinetics. While the majority of mIPSCs in these cells are mediated by α1-$GABA_ARs$, these newly induced currents had kinetic properties of α3-$GABA_ARs$, and, indeed, depended on expression of the α3 subunit in these cells, as tested in α3-KO mice (whereas deletion of the α1 subunit had no effect on this phenomenon). Along with evidence that the effects of ROS elevation are due to postsynaptic adaptations, rather than presynaptic changes in transmitter release, these results suggested that ROS activate a signaling cascade leading to the selective recruitment of α3-$GABA_AR$ to either "silent" synapses, or to *de novo* formed synaptic contacts from terminals known to form multiple release sites (Accardi *et al.*, 2014). In line with these results, insulin acutely increases $GABA_AR$ cell-surface expression in vitro (Wan *et al.*, 1997), but it is not established whether the same mechanism is involved.

Significance for GABAergic synapse plasticity: The main conclusion derived from studies of $GABA_AR$ (and gephyrin) post-translational regulation is that GABAergic synapses represent dynamic entities regulated by multiple mechanisms to homeostatically adjust the responsiveness and function of neuronal networks to changes in their environment. These regulatory adjustments concern both post- and extrasynaptic receptors and involve multiple intracellular signaling cascades. We have speculated elsewhere that by means of its role as scaffolding protein, gephyrin might interact with various effectors to adjust the structure and function of GABAergic synapses over a considerable dynamic

activity range (Tyagarajan & Fritschy, 2014); thereby ensuring homeostatic synaptic plasticity in mature neuronal circuits.

Posttranslational regulation of $GABA_A$Rs (and gephyrin) is implicated in functional plasticity of GABAergic synapses, as demonstrated in several systems (reviewed in (Kullmann *et al.*, 2012). For example, a well-studied model is rebound potentiation at inhibitory synapses of cerebellar Purkinje cells, which is induced by depolarization of Purkinje cells and involves Ca^{2+} influx and activation of calcium-calmodulin kinase 2 (Kano *et al.*, 1996). Rebound potentiation involves trafficking of $GABA_A$Rs to enhance their surface expression, and is necessary for adaptation of the vestibule-ocular reflex, a form of Purkinje cell-dependent motor learning (Kawaguchi & Hirano, 2007; Tanaka *et al.*, 2013). In addition to functional plasticity, regulation of $GABA_A$Rs also contributes to structural plasticity by inducing changes in GABAergic synaptic connectivity. For example, chronic treatment of Ts65Dn mouse mutants, a model of Down syndrome, with a selective α5-$GABA_A$R negative allosteric modulator reduced the density of GABAergic synapses in the hippocampal formation and normalized behavior in these mutants (Martínez-Cué *et al.*, 2013).

$GABA_A$ receptor heterogeneity in times of change: CNS development and adult neurogenesis

Developmental changes in $GABA_A$ receptor subunit expression: $GABA_A$Rs are expressed at early stages of fetal brain development by neural precursor cells and during neuronal differentiation, and have been proposed to regulate cell proliferation, migration, and differentiation, possibly through Ca^{2+}-mediated signals activated by neuronal depolarization (reviewed in (Represa & Ben-Ari, 2005; Cellot & Cherubini, 2013; Lu *et al.*, 2013)) (see box 4). Accordingly, one might expect developmental deficits in the CNS of $GABA_A$R subunit knock-out mice, notably with regard to subunits highly expressed in fetal brain. However, among the targeted deletions analyzed so far (α1-α6, β2, β3, δ, γ2) no detectable alterations in brain general architecture at birth have been reported, suggesting the existence of compensatory mechanisms substituting for the missing receptor subtype. Therefore, the absence of phenotype in these mutant mice should not be taken as evidence that $GABA_A$Rs are dispensable for regulating brain development. Rather, they might be so important that functional redundancy has been developed to prevent deleterious effects in case of dysfunction of a given subtype.

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* Box 4 approximately here *

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It is well established that the subunit composition of predominant $GABA_A$R subtypes changes during the period of synaptogenesis, accounting in part for the distinct functional and pharmacological

properties of $GABA_A$Rs in neonatal and mature brain (Fritschy *et al.*, 1994; Paysan *et al.*, 1997; Hutcheon *et al.*, 2000; Bosman *et al.*, 2002; Fagiolini *et al.*, 2004; Peden *et al.*, 2008; Hashimoto *et al.*, 2009). Nevertheless, there is only fragmentary information about the expression pattern of $GABA_A$R subunits in neural precursor cells and immature neurons. mRNA in situ hybridization data available in the Allen Brain Atlas (http://mouse.brain-map.org) show predominance of α2, α3, α5, β2, β3, γ2 subunits at E18.5. As this stage precedes synaptogenesis, one might assume that these receptors are mainly engaged in tonic, possibly depolarizing, GABAergic transmission. In particular, the α5 subunit is strongly expressed perinatally and decreases during synaptogenesis, except in regions where it remains abundant in adult brain (hippocampal formation, olfactory bulb, brainstem). Of note, down-regulation of the α5 subunit in layer 4 of the neocortex and its replacement by the α1 subunit, have been shown to be dependent on the integrity of thalamocortical projection (Paysan et al., 1997), providing an attractive model to study the mechanisms underlying (post-)transcriptional control of subunit expression. In contrast to α5, other subunits defining the main extrasynaptic $GABA_A$Rs of the adult brain (α4, α6, δ) are absent or expressed at low levels in fetal brain, and are upregulated when neuronal maturation nears completion. In human neocortex, the GABAergic system develops during the second half of pregnancy and infancy (Xu et al., 2011), and the expression of $GABA_A$R subunit genes appears to be coordinated based on their respective chromosomal localization (Fillman et al., 2010), with distinct up- and down regulation patterns suggestive of differential expression of specific $GABA_A$R subtypes.

To elucidate why the pattern of $GABA_A$R subunit expression evolves during ontogeny, considerable attention was given to α1-$GABA_A$Rs, which are upregulated in a region-specific manner during the phase of synaptogenesis to become the predominant $GABA_A$R subtype present in adult CNS (Fritschy *et al.*, 1994; Paysan *et al.*, 1994; Hashimoto *et al.*, 2009). As these receptors typically display fast decay kinetics and generate large amplitude events (Eyre *et al.*, 2012), their gradual appearance was taken as evidence for a maturation of GABAergic function (Vicini *et al.*, 2001; Bosman *et al.*, 2002); in particular to endow postsynaptic neurons with fast acting receptors matching the high firing rate of certain interneurons. Furthermore, as discussed in the next paragraph, α1-$GABA_A$Rs on perisomatic synapses of cortical pyramidal cells enable critical windows of plasticity (Fagiolini *et al.*, 2004). Similar, but reversible, changes in subunit expression have been proposed to account for plasticity of GABAergic transmission in the supraoptic nucleus during gestation and after delivery (Brussaard *et al.*, 1997). However, there is evidence from several electrophysiological studies that the acceleration of mIPSC kinetics occurring during brain development or following hormonal fluctuations do not depend solely on the subunit composition of $GABA_A$Rs (Koksma *et al.*, 2003; Koksma *et al.*, 2005; Peden *et al.*, 2008). Rather, post-translational modifications mechanisms affecting $GABA_A$R gating properties, and possibly their trafficking and interactions with scaffolding proteins, might also play a role in defining their gating properties.

To fully understand how differential expression of $GABA_A$Rs shapes the functional properties of GABAergic transmission, it will be essential to determine, as well, the developmental $GABA_A$R expression profile in interneurons, as these receptors will have a key role on the maturation and determine the firing properties of input cells controlling synaptic circuits.

$GABA_A$ receptor subtype setting critical periods of plasticity: The seminal observation that the time of opening of critical period windows (during which sensory deprivation causes lasting structural and functional alterations) can be delayed or advanced by reducing or enhancing GABAergic transmission provided direct evidence for the fundamental role played by $GABA_A$R-mediated transmission in regulating cortical development (Hensch *et al.*, 1998; Fagiolini & Hensch, 2000). Further investigations unambiguously showed that this effect requires a highly specific cortical circuit (Katagiri *et al.*, 2007), involving a central role for large parvalbumin-positive basket cells, which control the output of principal cells by activating α1-$GABA_A$Rs (reviewed in (Hensch, 2005)). The latter piece of the puzzle was brought about by the demonstration that diazepam is unable to advance the opening of a critical period window in α1(H101R) mice (Fagiolini *et al.*, 2004).

GABA is not the only neurotransmitter involved in this process, as modulation of nicotinic acetylcholine receptors by targeted deletion of Lynx1, a membrane-anchored prototoxin that negatively regulates nicotinic acetylcholine receptor function (Ibañez-Tallon *et al.*, 2002), allows re-opening of critical period windows in adulthood; remarkably, this effect of Lynx1 deletion can be blocked by co-application of diazepam, demonstrating that a balance between excitation and inhibition, rather than the action of a single transmitter, is determinant for setting the opening and closing of critical period windows (Morishita *et al.*, 2010).

The relevance of parvalbumin-positive basket cells for setting network configurations permissive for structural and functional plasticity, required for learning and memory acquisition, is not restricted to critical period windows, but appears to be a fundamental principle of brain plasticity (Donato *et al.*, 2013), involving a canonical pattern of interconnections between interneurons (Pfeffer *et al.*, 2013). According to these experiments, inhibitory control of parvalbumin-positive basket cells by VIP-positive interneurons is low in mice exposed to conditions permissive for learning (e.g., enriched environment) and high, either when a new task is acquired or when adverse conditions (e.g., fear conditioning) lead to memory retention. Remarkably, maturation and strength of inhibitory control of parvalbumin-positive basket cells is regulated by the transcription factor Otx2. This secreted molecule requires binding to a specific receptor in perineuronal nets – which selectively surround parvalbumin-positive interneurons – for cell penetration and activation of gene transcription (Beurdeley *et al.*, 2012).

Taken together, these results underscore the fact that GABAergic transmission in developing brain (and during permissive phases of plasticity critical for learning and circuit refinement) is regulated by

sophisticated mechanisms, and mediated by specific circuits containing defined $GABA_AR$ subtypes, such as α1-$GABA_ARs$ in synapses formed on principal cells by parvalbumin-positive basket cells.

Regulation of adult neurogenesis: In analogy to brain development, $GABA_ARs$ expressed by stem cells, neural precursor cells and immature neurons contribute to proliferation, migration, differentiation and synaptic integration of adult-born neurons (reviewed by (Overstreet *et al.*, 2005; Ge *et al.*, 2007; Sernagor *et al.*, 2010; Nissant & Pallotto, 2011)). Likewise, as in developing neurons, GABA initially exerts depolarizing effects on precursor cells (see Box 4), activating Ca^{2+}-dependent mechanisms that have enduring effects on precursor cell migration, cell survival, and subsequent neuronal maturation (Overstreet *et al.*, 2005; Ge *et al.*, 2006; Jagasia *et al.*, 2009; Chancey *et al.*, 2013).

Adult neurogenesis, taking place in the subventricular zone of the lateral ventricles and in the subgranular zone of the dentate gyrus, represents an important facet of brain plasticity regulated by GABAergic mechanisms. Experimentally, it offers an attractive paradigm for investigating the role of $GABA_AR$-mediated regulation of neuronal maturation and functional integration into pre-existing synaptic circuits. Thus, analyzing the consequences of $GABA_AR$ inactivation in radial glia-like stem cells revealed that local parvalbumin-positive fast-spiking interneurons in the dentate gyrus determine their mode of division (symmetric versus asymmetric) as well as neuronal versus glial fate (Song *et al.*, 2012); furthermore, the pharmacological profile of these receptors (zolpidem-insensitive) is compatible with expression of α5-$GABA_AR$ in these stem cells. Along the same line, investigating the role of extrasynaptic (α4, δ) and postsynaptic (α2) $GABA_ARs$ inactivated by gene targeting, we have provided evidence that these $GABA_AR$ subtypes regulate distinct phases of adult neurogenesis in the dentate gyrus (Duveau *et al.*, 2011). In line with findings that GABA exerts a negative control on neural precursor cell proliferation (Platel *et al.*, 2007), α4-KO mice exhibited increased neurogenesis. No phenotype was seen in δ-KO mice, as expected from the delayed expression of this subunit during ontogeny (but see (Whissell *et al.*, 2013)). Ablation of α4-$GABA_ARs$ also impaired dendritic growth and final positioning of adult-born granule cells; in contrast, α2-KO newborn neurons exhibited delayed pruning of dendritic branches, presumably to maintain inhibitory-excitatory balance upon maturation of glutamatergic inputs (Duveau *et al.*, 2011).

A more dramatic phenotype was observed upon selective α2 subunit inactivation in adult-born olfactory bulb granule cells (Pallotto *et al.*, 2012), which represent the main interneuron subtype of the olfactory bulb, continuously generated from mitotic precursor cells in the subventricular zone (Carleton *et al.*, 2003). As α2-$GABA_ARs$ provide most synaptic inhibition to these cells, their inactivation profoundly altered dendritic development, spine formation, and maturation of glutamatergic inputs. Also, modulation of dendritic differentiation by environmental enrichment or

deprivation, which is prominent in wildtype adult-born granule cells (Saghatelyan *et al.*, 2005), was abrogated in adult-born α2-KO granule cells (Pallotto *et al.*, 2012). The severity of these effects underscores the central role played by $GABA_AR$ in regulating neuronal differentiation. Moreover, these results taken together provide an exquisite demonstration that $GABA_AR$ subtypes are specialized to fulfill specific tasks, with considerable spatio-temporal specificity.

Significance for CNS disorders

Elucidation of the molecular organization and regulation of $GABA_AR$ subtypes opens new perspectives for understanding pathophysiological mechanisms in neurological and psychiatric diseases and for developing treatment approaches that go beyond symptomatic relief. Key aspects of the possible involvement of $GABA_AR$-mediated transmission in the pathophysiology of CNS disorders lie in their contribution to developmental processes and dependence on Cl^- and HCO_3^- fluxes (see Box 4). In turn, $GABA_AR$ dysfunction can be determined genetically, and/or depend on abnormal regulation and trafficking. Here, we briefly discuss these mechanisms at the light of a few selected examples.

$GABA_AR$ subunit mutations are typically associated with generalized, mostly idiopathic epilepsies and Dravet syndrome. They have been described in α subunits, β3, as well as γ2 and δ. While data are somewhat controversial, the majority of these mutations impair trafficking and cell surface expression of $GABA_ARs$, as well as their diffusion dynamics in the plasma membrane (hence, their postsynaptic clustering (Bouthour *et al.*, 2012)) (reviewed in (Galanopoulou, 2010; Macdonald *et al.*, 2010). Some of these effects have been proposed to be temperature-dependent, hence providing a plausible cause for febrile seizures. However, the general picture emerging from these studies is that there is that $GABA_AR$ mutations cause multiple molecular and biochemical alterations, which are not easily related to specific symptoms of disease, notably epileptogenesis and seizure occurrence; thereby reflecting the complexity of mechanisms underlying epileptic syndromes.

$GABA_AR$ mutations have also been associated with other CNS pathologies. For example, although the complex interactions between ethanol intoxication or ethanol dependence and $GABA_ARs$ go beyond the scope of this review, it is worth mentioning that a dominant point-mutation in the β1 subunit (L285R), which causes spontaneous channel openings and strongly enhances tonic inhibition in the nucleus accumbens, was shown recently to induce severe spontaneous ethanol consumption in mice (Anstee *et al.*, 2013). In the same study, the selective contribution of β1-containing $GABA_AR$ was confirmed by a second mutation, which caused similar behavioral phenotype. These data underscore the main contention of this review, namely how dysfunction of specific $GABA_AR$ subtypes, affecting a minor subpopulation of receptors, can cause strong behaviorally relevant effects.

Brain lesions, such as stroke or temporal lobe epilepsy with hippocampal sclerosis, lead to pathological alterations in GABAergic tonic inhibition, due to over-expression or reduction of specific $GABA_AR$ subtypes (reviewed in (Hines *et al.*, 2011; Grabenstatter *et al.*, 2012; Houser *et al.*, 2012)). In a seminal report investigating the relevance of tonic inhibition in stroke, Clarkson et al showed that reducing tonic inhibition in the peri-infarct area, a zone that is of crucial importance for functional recovery, with infusion of a benzodiazepine inverse agonist, or by genetically reducing expression of extrasynaptic $GABA_ARs$, promoted functional recovery (Clarkson *et al.*, 2010). These data go well in line with evidence that GABAergic transmission regulates neuronal plasticity by setting inhibitory-excitatory balance in neuronal networks.

$GABA_AR$-mediated transmission during brain development has been linked to the emergence of neurodevelopmental disorders, as well as adult-onset diseases that depend on proper formation of neuronal circuits, such as schizophrenia and depression (Luscher *et al.*, 2011b; Lewis, 2012; Marín, 2012). Similarly, abnormal GABAergic transmission during critical periods of development can cause severe sensory deficits, such as amblyopia, as well as impair sensory-motor and cognitive development; importantly, understanding the underlying mechanisms provides cues for therapeutic intervention (Bavelier *et al.*, 2010). Table 1 lists four principal mechanisms through which altered GABAergic transmission during CNS development and maturation has been implicated in brain diseases. In most cases, alterations can be traced back to mutations affecting neuronal maturation, synapse formation, and/or signaling cascades. As $GABA_AR$-mediated transmission and inhibitory-excitatory balance regulates key steps of neuronal migration and differentiation, the effects can be enduring. Thus, conditional inactivation of one γ2 subunit allele at defined stages of brain maturation induces either depression-like or anxiety-like behaviors in adult mice (Shen *et al.*, 2012). While the underlying mechanisms are not yet fully elucidated, they comprise alteration of adult neurogenesis in the dentate gyrus, as well as changes in synaptic connectivity and function of specific interneurons, notably fast-spiking parvalbumin-positive basket cells and somatostatin-positive interneurons. The crucial role played by interneurons for proper development of GABAergic synaptic connections is underscored by the long-ranging consequences of cell type-specific conditional gene deletions, such as inactivation of ErbB4 in parvalbumin-positive cortical interneurons, which affects formation of axo-axonic synapses and synchronization between prefrontal cortex and hippocampal formation, leading to schizophrenia-like phenotype (Del Pino *et al.*, 2013). In human, a corresponding deficit in axo-axonic synapses in prefrontal cortex is selectively found in schizophrenia but not bipolar disorder, and is accompanied by compensatory upregulation of the α2 subunit in the axon initial segment of pyramidal cells (reviewed in (Lewis & Hashimoto, 2007)).

The analysis of knock-in mice expressing diazepam-insensitive $GABA_AR$ subtypes (see Box 2) has noticeably expanded the catalogue of potential therapeutic applications of benzodiazepine site-ligands,

provided that subtype-specificity and differential efficacy can be achieved with novel compounds (reviewed in (Rudolph & Möhler, 2013)). These studies also implicate potential dysfunction of $GABA_A$Rs in a broader set of diseases than those treated with classical benzodiazepine site-ligands. Thus, α2-$GABA_A$Rs not only mediate diazepam anxiolysis, but they contribute to anxiety-related behaviors elicited by exposure to novelty and mild threat, as shown in α2-KO mice (Koester *et al.*, 2013). These receptors also contribute to mood disorders and chronic pain, and polymorphisms in *GABRA2* have been linked to alcohol dependence and drug abuse (Engin *et al.*, 2012). In chronic pain, the anti-hyperalgesic action of benzodiazepine site ligands devoid of sedative liability occurs primarily via stimulation of α2-$GABA_A$Rs in primary afferents and in the spinal cord dorsal horn, without involving supra-spinal sites (Witschi *et al.*, 2011; Paul *et al.*, 2013). α5-$GABA_A$Rs, as noted in Box 3, regulate learning and memory, as well as hippocampal neurogenesis, and represent a promising target for improving cognitive performance in Down syndrome patients. These receptors also have been implicated in memory deficits associated with acute neuroinflammation, possibly because interleukin 1β increases their cell surface expression in hippocampal neurons (Wang *et al.*, 2012). Considering the multiple post-translational mechanisms regulating $GABA_A$R-mediated transmission, the latter finding opens the possibility that multiple chemokines and cytokines might affect GABAergic transmission by activating the underlying signaling pathways. Therefore, one might speculate that $GABA_A$Rs contribute extensively to the mediation of neuro-immune interactions.

Conclusions and perspectives

This review discusses the evidence that $GABA_A$Rs form multiple subtypes, endowed with specific functional and pharmacological properties and being differentially regulated by multiple mechanisms, at the level of both gene expression and protein modification. Furthermore, we underscore that this regulation does not operate in isolation, but is intimately linked to the regulation of the postsynaptic scaffold organized by gephyrin, thereby vastly enlarging the repertoire of mechanisms that dynamically contribute to fine-tuning GABAergic transmission in response to various extracellular and intracellular signals. From this perspective, $GABA_A$R-mediated transmission appears as a multi-facetted process fundamental to proper brain development, function, and plasticity. The analysis of the specific role of $GABA_A$R subtypes reveals their implication in the pathophysiology of major CNS disorders and open novel perspectives for therapeutic intervention; notably based on subtype-specific ligands, and/or targeting specific signaling pathways regulating GABAergic synapse function.

While the concept of $GABA_A$R subtype, with well-defined subunit composition and functional properties, holds well in the adult brain, it is more difficult to define (and test) during brain development, when synaptic transmission is not yet present, and most effects of GABA are mediated by auto- or paracrine mechanisms. In particular, there are no behavioral readouts to probe the

consequences of altered $GABA_AR$ function in developing animals, and the significance of the major changes in subunit expression taking place during synaptogenesis remains unexplored. Nevertheless, the evidence available, in particular from studies of critical window plasticity and of adult neurogenesis (during which developmental processes are re-initiated in adult brain) provides strong support to the contention that $GABA_AR$ subtypes are tailor-made to modulate highly specific steps of neuronal differentiation and circuit formation during CNS ontogeny.

Several $GABA_AR$ subtypes, encoded by "rare" subunits ($\gamma1$, $\gamma3$, ε, π, τ), remain to be characterized. Their restricted localization in specific brain regions (notably hypothalamus and basal forebrain), coupled with a non-conventional pharmacological profile, offer opportunities for selective intervention to regulate specific brain functions, notably related to the neuro-endocrine axis, sleep-wake regulation, and central autonomic function. However, these distant perspectives will first require the development of analytical tools (and genetically engineered mice) to probe the function of these so far overlooked $GABA_AR$ subtypes.

The major focus given recently to "extrasynaptic" receptors, notably those containing the δ subunit, follows the same logic to exploit receptors possessing non-conventional pharmacological profile and unique regulatory mechanisms for improved therapeutic intervention. These perspectives are broad, ranging from stress-related disabilities to the treatment of stroke, epilepsy, alcohol intoxication, and drug dependency. However, much remains to be learned how these receptors are regulated and how they interact with membrane-proteins and intracellular effectors. The other major population of extrasynaptic $GABA_ARs$, containing the $\alpha5$ subunit, also offers promising perspectives as a target for improving intellectual disabilities, memory functions, and cognition. However, as seen with the analysis of $\alpha5$(H105R) mutant mice (see Box 3), care has to be taken in the interpretation of behavioral performance in mice.

The molecular heterogeneity of $GABA_AR$ subtypes provides the substrate for differential transcriptional and translational regulation. Much remains to be learned how post-translational regulation impacts on trafficking and function of specific receptor subtypes, as well as how the presence/absence of a defined subunit changes this regulation. One might speculate, for example, that the heterogeneity of β subunits, which are associated with multiple α subunits, adds to the regulation of $GABA_AR$ function, because the β subunits are differentially targeted by protein kinases and phosphatases (Houston *et al.*, 2008). In contrast, phosphorylation of the $\gamma2$ subunit might represent a mechanism common to multiple $GABA_AR$ subtypes. Our recent observation that $\alpha3$-, but not $\alpha1$-$GABA_ARs$, are selectively targeted to postsynaptic sites to enhance GABAergic transmission when intracellular levels of ROS are increased (Accardi *et al.*, 2014) provides a striking example for a $GABA_AR$ subtype-specific regulation to adjust the strength of inhibition in response to a specific

stimulus. Uncovering the underlying mechanisms of this specific adaptation will help understanding the difference between α3- versus α1-$GABA_A$Rs. Furthermore, considering that α1-, α2, and α3-$GABA_A$Rs comprise the vast majority of postsynaptic $GABA_A$Rs, it will be essential to unravel their distinguishing features, which require them to be differentially expressed and targeted to distinct subcellular sites. As a first step towards this goal, a proteomics analysis of GABAergic PSDs, and/or the characterization of the interactome of each main $GABA_A$R subtype, would provide an exhaustive list of signaling pathways involved in their trafficking and synaptic function.

Acknowledgments

The work of JMF is supported by the Swiss National Science Foundation.

List of abbreviations

DGC	dystrophin-glycoprotein complex
$GABA_AR$	$GABA_A$ receptor
GlyR	Glycine receptor
NLGN	Neuroligin gene
NL2	Neuroligin2
PSD	Postsynaptic density
ROS	radical oxygen species

Box 1: Limitations of immunohistochemistry for studying $GABA_A$ receptors

Whereas $GABA_AR$ are pentameric protein complexes, immunohistochemistry only allows the visualization of individual subunits, each of which belongs to several subtypes. Furthermore, as the pentamer contains 2α and 2β subunits, it is not possible in neurons expressing multiple α or β subunit variants to determine whether they belong to the same receptor, or to distinct receptors with similar subcellular distribution. Therefore, the exact $GABA_AR$ subtype repertoire of any neuron type is not established (see, for example, (Panzanelli *et al.*, 2011)). A further limitation is that, so far, there are no antibodies suitable for immunochemistry for some of the $GABA_AR$ subunits. In particular, very little information is available for the distribution of the γ1, γ3, ε, π, and τ subunit proteins, as well as ρ1-ρ3 subunits, either at a regional, cellular, or subcellular level. As a result, several $GABA_AR$ subtypes, probably with atypical pharmacology and a highly selective distribution, remain poorly characterized and their contribution to inhibitory transmission in these specific CNS areas is unknown.

Besides these limitations, it should be emphasized that although immunohistochemistry reveals the detailed distribution and relative staining intensity of each subunit across the CNS, this method does not allow quantitative comparisons between antibodies (and thus assessing the abundance of a given subunit relative to other subunits), because the affinity of antibodies to their epitope, and its accessibility in tissue sections prepared for histology, cannot be directly measured. Furthermore, in the light of evidence showing that the specificity and sensitivity of the immunohistochemical procedure critically depends on epitope accessibility (Lorincz & Nusser, 2008), each antibody should be tested with various antigen retrieval methods. This is particularly important to interpret lack of staining for a specific antibody in some regions and to accurately determine the subcellular distribution of $GABA_ARs$.

This latter point is of crucial relevance for understanding the functional organization of the GABAergic system. Functional and biochemical studies differentiate among several pools of $GABA_ARs$ in neurons, being localized intracellularly (reflecting biosynthesis, metabolism, reserve pool) or at the cell surface, post- or extrasynaptically (see Box 3). Immunohistochemical studies have shown that the detection of postsynaptic $GABA_ARs$ is largely impaired by aldehyde fixation and requires alternative methods to the classical transcardial perfusion. Therefore, immunohistochemical analysis of $GABA_AR$ subunit distribution provides distinct results depending on the fixation method used. Furthermore, we had noted in our initial report (Fritschy & Mohler, 1995) that the cellular distribution pattern of $GABA_AR$ revealed by each antibody across brain regions is highly variable, ranging from diffuse staining of the neuropil to “Golgi-like” staining of a few neurons, outlining their entire dendritic tree. This feature makes it impossible to quantify with a single densitometric parameter the abundance of a subunit in a given brain region, as it can be widely different for various cell types.

Therefore, analyses of regional distribution patterns, showing marked differences in subunit abundance across different anatomical structures of the CNS, need to be complemented with methods allowing cellular and subcellular resolution to map $GABA_AR$ subtypes in a synapse-specific manner, and to derive some quantitative measurement of relative receptor abundance. We have recently reported a method allowing concurrent visualization of postsynaptic $GABA_ARs$ with high sensitivity and resolution and biochemical analysis using brain tissue from the same animal (Notter *et al.*, 2013). In

the future, new methods will have to be devised to unequivocally detect pre-synaptic $GABA_A$Rs, whose existence is well established functionally, but whose distribution in axons remains largely elusive.

The limitations of immunohistochemistry are even more evident for ultrastructural studies, in which the sensitivity of the method is reduced by the strong fixation required for ultrastructural preservation. It was recognized early on that pre-embedding immune-electron microscopy is not suitable for the detection of $GABA_A$Rs at postsynaptic sites (Somogyi *et al.*, 1989). Post-embedding techniques, notably following tissue embedding in Lowicryl permitted to circumvent this problem, but have low sensitivity and have been successful with only a limited number of subunits (Nusser *et al.*, 1995; Somogyi *et al.*, 1996; Panzanelli *et al.*, 2004). More recently, the development of SDS-digestion after freeze-fracture has enabled substantial progress by allowing the 3-D visualization and quantification of $GABA_A$Rs in the plasma membrane (Kasugai *et al.*, 2010), albeit working only with a limited number of antibodies.

Box 2: Lessons from *Gabra* knock-out and knock-in mice: $GABA_A$ receptor subtypes fulfill specific tasks

Targeted deletion of a $GABA_AR$ subunit gene, especially when constitutive, can lead to important changes in the distribution and expression pattern of the remaining subunits, suggestive of compensatory adaptations. For example, *Gabra1* knock-out (α1-KO) mice exhibit upregulation of α2- and α3-$GABA_ARs$ in regions where the α1 subunit is abundant (Kralic et al., 2006; Zeller et al., 2008); δ-KO mice exhibit increased α4 subunit expression, associated with the γ2 subunit, and with altered subcellular distribution (Peng et al., 2002). Typically, however, the receptor subtype that is missing as a consequence of the deletion is not merely "replaced" by another subtype present in the same cell. This feature is particularly striking in neurons expressing a mixture of postsynaptic and extrasynaptic receptors. Deletion of the α subunit variant present in the postsynaptic receptors leads to their disappearance (and corresponding loss of postsynaptic currents), whereas the extrasynaptic receptors remain either unchanged, or increased (Kralic et al., 2006; Peden et al., 2008). Therefore, the inability of α4-$GABA_ARs$ to cluster at postsynaptic sites is not due to competition with other receptor subtypes. In neurons expressing multiple postsynaptic receptors, synapse-specific rearrangements occur, but there is no replacement of the missing receptor, as seen, for example, in CA1 pyramidal cells of α2-KO mice, where α1-$GABA_ARs$ remain unaffected in perisomatic synapses, but disappear from the axon initial segment (Panzanelli et al., 2011). Some striking forms of compensation have been reported, which remain unexplained. Thus, in thalamic reticular neurons of α3-KO mice, immunohistochemistry reveals apparent loss of postsynaptic $GABA_ARs$ and gephyrin, but these mutants exhibit larger postsynaptic currents than wildtype mice (Schofield et al., 2009).

These observations lend to the contention that $GABA_AR$ subtypes, defined by their subunit composition, are unique functional entities, fulfilling specific tasks, without being inter-changeable within a given type of neuron. This contention received further support from the analysis of (H101R) knock-in mice, engineered to remove the diazepam binding site located at the α/γ interface of the pentameric complex, without affecting assembly, cell surface trafficking, regulation, and gating of the receptor (Rudolph *et al.*, 1999; Löw *et al.*, 2000; Crestani *et al.*, 2002; Yee *et al.*, 2005). Behavioral analysis of H/R knock-in mice for each the four α subunit variants assembled in diazepam-sensitive $GABA_ARs$ revealed loss of specific effects of diazepam, which allowed to classify the contribution of each subtype to the spectrum of diazepam's effects. Thus, sedation only involves α1-$GABA_ARs$, whereas anxiolysis occurs upon allosteric modulation of α2-$GABA_ARs$, and when stress is involved, partially α3-$GABA_ARs$ (reviewed in (Rudolph & Möhler, 2004)). More recent studies have shown a corresponding segregation of other effects of diazepam (and midazolam), including benzodiazepine addiction (Tan *et al.*, 2010), tachypnea (Masneuf *et al.*, 2012), and anti-hyperalgesia (Knabl *et al.*, 2008), to specific $GABA_AR$ subtypes. Importantly, electrophysiological analyses confirmed that the point-mutations are functionally silent. However, in α5(H105R)-mutants, it leads to decreased expression of α5-$GABA_ARs$, which is behaviorally significant (Prut *et al.*, 2010) (see Box 3).

A distinction of $GABA_AR$ subtypes based on the β subunit variants is less straightforward, in particular because each β subunit can be associated with various a subunits. Nevertheless, β3 subunit-containing $GABA_ARs$ selectively mediate the action of intravenous general anesthetics, as well as part of the effects of pentobarbital, as shown in β3(N265M) mutant mice (Jurd *et al.*, 2003; Zeller *et al.*, 2007). In addition, neuron-specific deletion of these receptors curtails survival beyond early postnatal age in the majority of mutant mice (Ferguson *et al.*, 2007).

Taken together, these findings are of fundamental relevance not only for the development of efficacious benzodiazepine site-ligands devoid of unwanted side-effects (in particular, sedation), but also to investigate how neuronal circuits are being assembled during brain development and regulated by plasticity mechanisms in adulthood.

Box 3: Extrasynaptic $GABA_A$ receptors

Tonic inhibition, mediated by persistent activation of extrasynaptic $GABA_A$Rs, is an important determinant of neuronal excitability and is increasingly recognized to play a key role in mediating effects of neurosteroids, as well as to contribute to pathophysiology of major disease states (reviewed in (Belelli *et al.*, 2009; Gunn *et al.*, 2011; Brickley & Mody, 2012)). While tonic inhibition is widely considered to reflect receptor activation by ambient GABA, this vision has been questioned by evidence that spontaneous openings of $GABA_A$Rs might contribute to most of tonic currents that can be recorded in dentate gyrus granule cells (Wlodarczyk et al., 2013). Extrasynaptic receptors containing the δ subunit have a very high affinity to GABA, and mediate most actions of neurosteroids, and thus contribute to regulate brain activity under circumstances when their synthesis is increased, including stress, delivery, ethanol intoxication (Sarkar *et al.*, 2011; Carver & Reddy, 2013). These receptors are selectively modulated by the super-agonist gaboxadol (but insensitive to classical benzodiazepine agonists) (Mortensen *et al.*, 2010). Until recently, it was unclear how the expression and cell surface expression of extrasynaptic receptors is regulated. Evidence now indicates that tonic inhibition in the dentate gyrus and thalamus is modulated by PKA and PKC activity (targeting α4-$GABA_A$R) (Connelly *et al.*, 2013a), for example upon stimulation of $GABA_B$ receptors (Connelly et al., 2013b; Tao et al., 2013). Specifically, PKC-mediated phosphorylation of Ser443 in the α4 subunit was shown to enhance cell surface expression and activity of these receptors (Abramian *et al.*, 2010). However, another study contends that PKC activation reduces tonic inhibition in the thalamus by targeting the β2 subunit (Bright & Smart, 2013). As tonic inhibition is a major determinant of neuronal excitability, these data unravel novel, albeit contradictory, mechanisms that potentially have major effects on network activity.

Besides α4/β/δ receptors, α5-$GABA_A$Rs (most probably composed of α5/β3/γ2 subunits) also form a prominent population of extrasynaptic receptors in the hippocampal formation, olfactory bulb, and cerebral cortex. These receptors are modulated by diazepam, but insensitive to zolpidem. Interest in these receptors was triggered by the observations in α5(H105R)-mutant mice (carrying diazepam-insensitive α5-$GABA_A$Rs, see Box 2) that they do not develop tolerance to the sedative (i.e., motor impairing) action of diazepam (van Rijnsoever *et al.*, 2004). Furthermore, α5(H105R)-mutant mice exhibited ~30% reduction in α5-$GABA_A$Rs but displayed improved performance in a hippocampus-dependent memory task (trace fear conditioning) compared to wildtype mice (Crestani *et al.*, 2002). This observation opened the tantalizing perspective that reducing the function of α5-$GABA_A$Rs might be exploited to reverse disease-related deficits in cognition and memory performance, and triggered the search for inverse agonists acting selectively at these receptors (reviewed in (Rudolph & Möhler, 2013)). However, a more systematic analysis of α5(H105R)-mutant mice revealed increased basal locomotion and altered memory for location of objects, indicative of hippocampal dysfunction (Prut *et al.*, 2010). Therefore, the reduced expression of α5-$GABA_A$R induces complex bidirectional changes in behavioral performance in these mutants.

Other receptors, presumably containing the γ2 subunit, as revealed by their sensitivity to diazepam, are located extrasynaptically and mediate tonic inhibition. They include in particular α3-$GABA_AR$ in the basolateral amygdala (Marowsky *et al.*, 2012) and the inferior olivary nucleus (Devor *et al.*, 2001). The rules governing the extrasynaptic localization of these receptors are not understood. The majority of α3-$GABA_ARs$, notably in thalamic reticular neurons or in hippocampal or cerebellar interneurons, form postsynaptic clusters associated with gephyrin (Studer *et al.*, 2006; Schneider Gasser *et al.*, 2007; Notter *et al.*, 2013). Whereas interactions with gephyrin are thought to be crucial for postsynaptic receptors, other mechanisms remaining to be explored, might supersede them to determine (and maintain) α3-$GABA_ARs$ at extrasynaptic sites in specific neuron populations. A similar dichotomy also exist for α5-$GABA_ARs$, which are not strictly extrasynaptic in the hippocampal formation (Serwanski *et al.*, 2006); those located extrasynaptically were shown to interact with radixin, a phospho-protein belonging to the ezrin-radixin-meosin protein family and interacting with the actin cytoskeleton (Loebrich *et al.*, 2006).

Box 4: $GABA_A$ receptor-mediated "excitation"

$GABA_A$Rs being selectively permeable for Cl^- and HCO_3^- ions (Kaila, 1994), there is now a large consensus that elevation of the intracellular concentration of either species might result in a depolarizing current upon $GABA_A$R activation, and thereby potentially "excite" this neuron (Blaesse *et al.*, 2009). There is ample evidence for $GABA_A$R-mediated depolarization of immature (and mature) neurons and NG2 cells, leading to Ca^{2+}-influx; opening the door to speculating about the roles of Ca^{2+} as second messenger in these cells, in particular to control the cell cycle and differentiation mechanisms (Tanaka *et al.*, 2009; Merz *et al.*, 2011; Young *et al.*, 2012). It has also been proposed that $GABA_A$R-mediated excitation precedes (and is replaced by) glutamatergic transmission during maturation of cortical neurons (Hennou *et al.*, 2002), and that "excitatory" GABA drives giant depolarizing potentials, which are network phenomena thought to contribute to proper axonal wiring of the developing CNS (reviewed in (Dehorter *et al.*, 2012)).

The reality of $GABA_A$R-mediated excitation *in vivo* has been much debated, as well as its functional significance and the main ion species responsible for it. In particular, there is often confusion about the excitatory effects of depolarizing GABA. Considering that one of the main effect of $GABA_A$R activation is a net increase in membrane conductance, opposing the depolarizing effect of positive charge influx induced by any other neurotransmitter. Therefore, while a neuron can be depolarized by GABA, this does not necessarily translate as being "excited".

The prevalent view is that GABA-induced depolarization is due to high expression of the co-transporter NKCC1 and low expression of KCC2, which exert opposing action on intracellular Cl^-. Nevertheless, in mature neurons (expressing high levels of KCC2), intense $GABA_A$R stimulation can lead eventually to neuronal depolarization due to KCC2-mediated K^+ efflux (Viitanen *et al.*, 2010). This biphasic response, which involves short-lasting changes in ionic driving force of $GABA_A$R and reduces the efficacy of diazepam (Deeb *et al.*, 2013), has been coined as "short-term ionic plasticity" (Raimondo *et al.*, 2012). Despite the importance of NKCC1 and KCC2 for regulating GABA function, in particular under pathological conditions such as epilepsy and chronic pain, little is known about their precise subcellular localization and functional regulation. Evidence is now emerging that transcriptional and post-translational mechanisms, involving among others BDNF signaling, have major impact on the availability and cell surface expression of KCC2 (Yeo *et al.*, 2009; Lee *et al.*, 2011; Puskarjov *et al.*, 2012; Chamma *et al.*, 2013; Sun *et al.*, 2013). Furthermore, it is now being recognized that, besides NKCC1 and KCC2, the developmental maturation of carbonic anhydrases is a major determinant of the driving force of $GABA_A$Rs in immature brain (Rivera *et al.*, 2005). Thus, absence of carbonic anhydrase (upon targeted gene deletion) enhances depolarizing action of GABA and induces seizures in neonatal mice (Ruusuvuori *et al.*, 2013). Finally, it should be emphasized that, unlike initial speculations that the depolarizing and hyperpolarizing effects of GABA might be mediated by distinct $GABA_A$R subtypes, there is no evidence supporting this possibility. These speculations were triggered, in part, by observations that the subunit composition of major $GABA_A$R subtypes changes drastically, in particular in neocortex and thalamus, during the phase of synaptogenesis (see main text). However,

this subunit switch appears to be unrelated to GABA depolarization, and its significance remains matter of speculation.

References

Abramian, A.M., Comenencia-Ortiz, E., Vithlani, M., Tretter, E.V., Sieghart, W., Davies, P.A. & Moss, S.J. (2010) Protein kinase C phosphorylation regulates membrane insertion of $GABA_A$ receptor subtypes that mediate tonic inhibition. *J. Biol. Chem.*, **285**, 41795-41805.

Accardi, M., Daniels, B., Brown, P., Fritschy, J., Tyagarajan, S. & Bowie, D. (2014) Mitochondrial reactive oxygen species regulate the strength of inhibitory GABA-mediated synaptic transmission. *Nature Commun*, **in press.**

Anstee, Q., Knapp, S., Maguire, E., Hosie, A., Thomas, P., Mortensen, M., Bhome, R., Martinez, A., Walker, S., Dixon, C., Ruparelia, K., Montagnese, S., Kuo, Y., Herlihy, A., Bell, J., Robinson, I., Guerrini, I., McQuillin, A., Fisher, E., Ungless, M., Gurling, H., Morgan, M., Brown, S., Stephens, D., Belelli, D., Lambert, J., Smart, T. & Thomas, H. (2013) Mutations in the Gabrb1 gene promote alcohol consumption through increased tonic inhibition. *Nat Commun*, **4**, 2816.

Balic, E., Rudolph, U., Fritschy, J.M., Mohler, H. & Benke, D. (2009) The α5(H105R) mutation impairs α5 selective binding properties by altered positioning of the α5 subunit in $GABA_A$ receptors containing two distinct types of α subunits. *J. Neurochem.*, **110**, 244-254.

Bannai, H., Lévi, S., Schweizer, C., Inoue, T., Launey, T., Racine, V., Sibarita, J.B., Mikoshiba, K. & Triller, A. (2009) Activity-dependent tuning of inhibitory neurotransmission based on $GABA_A$R diffusion dynamics. *Neuron*, **62**, 670-682.

Bavelier, D., Levi, D., Li, R., Dan, Y. & Hensch, T. (2010) Removing brakes on adult brain plasticity: from molecular to behavioral interventions. *J Neurosci*, **30**, 14964-14971.

Belaidi, A. & Schwarz, G. (2013) Metal insertion into the molybdenum cofactor: product-substrate channelling demonstrates the functional origin of domain fusion in gephyrin. *Biochem J*, **450**, 149-157.

Belelli, D., Harrison, N., Maguire, J., Macdonald, R., Walker, M. & Cope, D. (2009) Extrasynaptic $GABA_A$ receptors: form, pharmacology, and function. *J Neurosci*, **29**, 12757-12763.

Belelli, D. & Lambert, J.J. (2005) Neurosteroids: endogenous regulators of the $GABA_A$ receptor. *Nature Rev. Neurosci.*, **6**, 565-575.

Ben-Ari, Y. (2002) Excitatory actions of GABA during development: The nature of the nurture. *Nature Rev. Neurosci.*, **3**, 728-739.

Beurdeley, M., Spatazza, J., Lee, H., Sugiyama, S., Bernard, C., Di Nardo, A., Hensch, T. & Prochiantz, A. (2012) Otx2 binding to perineuronal nets persistently regulates plasticity in the mature visual cortex. *J Neurosci*, **32**, 9429-9437.

Blaesse, P., Airaksinen, M.S., Rivera, C. & Kaila, K. (2009) Cation-chloride cotransporters and neuronal function. *Neuron*, **61**, 820-838.

Blundell, J., Tabuchi, K., Bolliger, M.F., Blaiss, C.A., Brose, N., Liu, X., Südhof, T.C. & Powell, C.M. (2009) Increased anxiety-like behavior in mice lacking the inhibitory synapse cell adhesion molecule neuroligin 2. *Genes Brain & Behavior*, **8**, 114-126.

Bogdanov, Y., Michels, G., Armstrong-Gold, C., Haydon, P., Lindstrom, J., Pangalos, M. & Moss, S. (2006) Synaptic $GABA_A$ receptors are directly recruited from their extrasynaptic counterparts. *EMBO J*, **25**, 4381-4389.

Boileau, A.J., Pearce, R.A. & Czajkowsk, C. (2005) Tandem subunits effectively constrain $GABA_A$ receptor stoichiometry and recapitulate receptor kinetics but are insensitive to $GABA_A$ receptor-associated protein. *J Neurosci*, **25**, 11219-11230.

Bolliger, M.F., Frei, K., Winterhalter, K.H. & Gloor, S.M. (2001) Identification of a novel neuroligin in humans which binds to PSD-95 and has a widespread expression. *Biochem J.* , **356**, 581-588.

Bosman, L.W.J., Rosahl, T.W. & Brussaard, A.B. (2002) Neonatal development of the rat visual cortex: synaptic function of $GABA_A$ receptor α subunits. *J. Physiol.*, **545**, 169-181.

Bouthour, W., Leroy, F., Emmanuelli, C., Carnaud, M., Dahan, M., Poncer, J. & Lévi, S. (2012) A human mutation in Gabrg2 associated with generalized epilepsy alters the membrane dynamics of $GABA_A$ receptors. *Cereb Cortex*, **22**, 1542-1553.

Brickley, S.G. & Mody, I. (2012) Extrasynaptic $GABA_A$ receptors: their function in the CNS and implications for disease. *Neuron*, **12**, 23-34.

Bright, D. & Smart, T. (2013) Protein kinase C regulates tonic $GABA_A$ receptor-mediated inhibition in the hippocampus and thalamus. *Eur J Neurosci*, **Epub ahead of print**.

Brünig, I., Penschuck, S., Berninger, B., Benson, J.A. & Fritschy, J.M. (2001) BDNF reduces miniature inhibitory postsynaptic currents by rapid down-regulation of $GABA_A$ receptor surface expression. *Eur. J. Neurosci.*, **13**, 1320-1328.

Brussaard, A.B., Kits, K.S., Baker, R.E., Willems, W.P.A., Leyting-Vermeulen, J.W., Voorn, P., Smit, A.B., Bicknell, R.J. & Herbison, A.E. (1997) Plasticity in fast synaptic inhibition of adult

oxytocin neurons caused by switch in $GABA_A$ receptor subunit expression. *Neuron*, **19**, 1103-1114.

Budreck, E.C. & Scheiffele, P. (2007) Neuroligin-3 is a neuronal adhesion protein at GABAergic and glutamatergic synapses. *Eur. J. Neurosci.*, **26**, 1738-1748.

Cajigas, I., Tushev, G., Will, T., tom Dieck, S., Fuerst, N. & Schuman, E. (2012) The local transcriptome in the synaptic neuropil revealed by deep sequencing and high-resolution imaging. *Neuron*, **74**, 453-466.

Carleton, A., Petreanu, L.T., Lansord, R., Alvarez-Buylla, A. & Lledo, P.M. (2003) Becoming a new neuron in the adult olfactory bulb. *Nature Neurosci.*, **6**, 507-518.

Carver, C. & Reddy, D. (2013) Neurosteroid interactions with synaptic and extrasynaptic $GABA_A$ receptors: regulation of subunit plasticity, phasic and tonic inhibition, and neuronal network excitability. *Psychopharmacol.*, **230**, 151-188.

Cellot, G. & Cherubini, E. (2013) Functional role of ambient GABA in refining neuronal circuits early in postnatal development. *Front Neural Circuits*, **7**, 136.

Chamma, I., Heubl, M., Chevy, Q., Renner, M., Moutkine, I., Eugène, E., Poncer, J. & Lévi, S. (2013) Activity-dependent regulation of the K/Cl transporter KCC2 membrane diffusion, clustering, and function in hippocampal neurons. *J Neurosci*, **33**, 15488-15503.

Chancey, J., Adlaf, E., Sapp, M., Pugh, P., Wadiche, J. & Overstreet-Wadiche, L. (2013) GABA depolarization is required for experience-dependent synapse unsilencing in adult-born neurons. *J Neurosci*, **33**, 6614-6622.

Chandra, D., Jia, F., Liang, J., Peng, Z., Suryanarayanan, A., Werner, D.F., Spigelman, I., Houser, C.R., Olsen, R.W., Harrison, N.L. & Homanics, G.E. (2006) $GABA_A$ receptor $\alpha4$ subunits mediate extrasynaptic inhibition in thalamus and dentate gyrus and the action of gaboxadol. *Proc. Natl. Acad. Sci. USA*, **103**, 15230-15235.

Chen, A., Nguyen, C., Copenhagen, D., Badurek, S., Minichiello, L., Ranscht, B. & Reichardt, L. (2011) TrkB (tropomyosin-related kinase B) controls the assembly and maintenance of GABAergic synapses in the cerebellar cortex. *J Neurosci*, **31**, 2769-2780.

Chih, B., Engelman, H. & Scheiffele, P. (2005) Control of excitatory and inhibitory synapse formation by neuroligins. *Science*, **307**, 1324-1328.

Chiou, T.T., Bonhomme, B., Jin, H., Miralles, C.P., Xiao, H., Fu, Z., Harvey, R.J., Harvey, K., Vicini, S. & De Blas, A.L. (2011) Differential regulation of the postsynaptic clustering of γ-

aminobutyric acid type A ($GABA_A$) receptors by collybistin isoforms. *J. Biol. Chem.*, **286**, 22456-22468.

Christian, C., Herbert, A., Holt, R., Peng, K., Sherwood, K., Pangratz-Fuehrer, S., Rudolph, U. & Huguenard, J. (2013) Endogenous positive allosteric modulation of $GABA_A$ receptors by diazepam binding inhibitor. *Neuron*, **78**, 1063-1074.

Clarkson, A., Huang, B., Macisaac, S., Mody, I. & Carmichael, S. (2010) Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. *Nature*, **468**, 305-309.

Connelly, W., Errington, A., Giovanni, G. & Crunelli, V. (2013a) Metabotropic regulation of extrasynaptic $GABA_A$ receptors. *Front Neural Circuits*, **7**, 171.

Connelly, W., Fyson, S., Errington, A., McCafferty, C., Cope, D., Di Giovanni, G. & Crunelli, V. (2013b) $GABA_B$ Receptors Regulate Extrasynaptic $GABA_A$ Receptors. *J Neurosci*, **33**, 3780-3785.

Crestani, F., Keist, R., Fritschy, J.M., Benke, D., Vogt, K., Prut, L., Bluethmann, H., Mohler, H. & Rudolph, U. (2002) Trace fear conditioning involves hippocampal $\alpha5$ $GABA_A$ receptors. *Proc. Natl. Acad. Sci. USA*, **99**, 8980-8985.

Deeb, T., Nakamura, Y., Frost, G., Davies, P. & Moss, S. (2013) Disrupted Cl^- homeostasis contributes to reductions in the inhibitory efficacy of diazepam during hyperexcited states. *Eur J Neurosci*, **38**, 2453-2467.

Dehorter, N., Vinay, L., Hammond, C. & Ben-Ari, Y. (2012) Timing of developmental sequences in different brain structures: physiological and pathological implications. *Eur J Neurosci*, **35**, 1846-1856.

Del Pino, I., García-Frigola, C., Dehorter, N., Brotons-Mas, J., Alvarez-Salvado, E., Martínez de Lagrán, M., Ciceri, G., Gabaldón, M., Moratal, D., Dierssen, M., Canals, S., Marín, O. & Rico, B. (2013) Erbb4 deletion from fast-spiking interneurons causes schizophrenia-like phenotypes. *Neuron*, **79**, 1152-1168.

Devor, A., Fritschy, J.M. & Yarom, Y. (2001) Spatial distribution and subunit composition of $GABA_A$ receptors in the inferior olivary nucleus. *J. Neurophysiol.*, **85**, 1686-1696.

Dieni, C., Chancey, J. & Overstreet-Wadiche, L. (2013) Dynamic functions of GABA signaling during granule cell maturation. *Front Neural Circuits*, **6**, 113.

Donato, F., Rompani, S. & Caroni, P. (2013) Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. *Nature*, **504**, 272-276.

Dong, N., Qi, J.S. & Chen, G. (2007) Molecular reconstitution of functional GABAergic synapses with expression of neuroligin-2 and $GABA_A$ receptors. *Mol. Cell. Neurosci.*, **35**, 14-23.

Duveau, V., Laustela, S., Barth, L., Gianolini, F., Vogt, K.E., Keist, R., Chandra, D., Homanics, G.E., Rudolph, U. & Fritschy, J.M. (2011) Spatiotemporal specificity of $GABA_A$ receptor-mediated regulation of adult hippocampal neurogenesis. *Eur. J. Neurosci.*, **34**, 362-373.

Ehninger, D., Li, W., Fox, K., Stryker, M. & Silva, A. (2008) Reversing neurodevelopmental disorders in adults. *Neuron*, **60**, 950-960.

Engin, E., Liu, J. & Rudolph, U. (2012) α2-containing $GABA_A$ receptors: a target for the development of novel treatment strategies for CNS disorders. *Pharmacol Ther*, **136**, 142-152.

Essrich, C., Lorez, M., Benson, J.A., Fritschy, J.M. & Luscher, B. (1998) Postsynaptic clustering of major $GABA_A$ receptor subtypes requires the γ2 subunit and gephyrin. *Nature Neurosci.*, **1**, 563-571.

Eyre, M., Renzi, M., Farrant, M. & Nusser, Z. (2012) Setting the time course of inhibitory synaptic currents by mixing multiple $GABA_A$ receptor α subunit isoforms. *J Neurosci*, **32**, 5853-5867.

Fagiolini, M., Fritschy, J.M., Löw, K., Möhler, H., Rudolph, U. & Hensch, T.K. (2004) Specific $GABA_A$ circuits for visual cortical plasticity. *Science*, **303**, 1681-1683.

Fagiolini, M. & Hensch, T. (2000) Inhibitory threshold for critical-period activation in primary visual cortex. *Nature*, **404**, 183-186.

Fang, C., Deng, L., Keller, C.A., Fukata, M., Fukata, Y., Chen, G. & Luscher, B. (2006) GODZ-mediated palmitoylation of $GABA_A$ receptors is required for normal assembly and function of GABAergic inhibitory synapses. *J. Neurosci.*, **26**, 12758-12768.

Farrant, M. & Nusser, Z. (2005) Variations on an inhibitory theme: Phasic and tonic activation of $GABA_A$ receptors. *Nature Rev. Neurosci.*, **6**, 215-229.

Feng, G., Tintrup, H., Kirsch, J., Nichol, M.C., Kuhse, J., Betz, H. & Sanes, J.R. (1998) Dual requirement for gephyrin in glycine receptor clustering and molybdoenzyme activity. *Science*, **282**, 1321-1324.

Ferguson, C., Hardy, S.L., Werner, D.F., Hileman, S.M., Delorey, T.M. & Homanics, G.E. (2007) New insight into the role of the β3 subunit of the $GABA_A$-R in development, behavior, body weight regulation, and anesthesia revealed by conditional gene knockout. *BMC Neuroscience*, **8**, 85.

Fillman, S.G., Duncan, C.E., Webster, M.J., Elashoff, M. & Weickert, C.S. (2010) Developmental co-regulation of the β and γ $GABA_A$ receptor subunits with distinct α subunits in the human dorsolateral prefrontal cortex. *Int. J. Dev. Neurosci.*, **28**, 513-519.

Fritschy, J.M., Harvey, R.J. & Schwarz, G. (2008) Gephyrin, where do we stand, where do we go? *Trends Neurosci.*, **31**, 257-264.

Fritschy, J.M. & Mohler, H. (1995) $GABA_A$-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *J. Comp. Neurol.*, **359**, 154-194.

Fritschy, J.M., Panzanelli, P., Kralic, J.E., Vogt, K.E. & Sassoè-Pognetto, M. (2006) Differential dependence of axo-dendritic and axo-somatic GABAergic synapses on $GABA_A$ receptors containing the α1 subunit in Purkinje cells. *J. Neurosci.*, **26**, 3245-3255.

Fritschy, J.M., Panzanelli, P. & Tyagarajan, S.K. (2012) Molecular and functional heterogeneity of GABAergic synapses. *Cell Mol Life Sci.*, **69**, 2485-2499.

Fritschy, J.M., Paysan, J., Enna, A. & Mohler, H. (1994) Switch in the expression of rat $GABA_A$-receptor subtypes during postnatal development: an immunohistochemical study. *J. Neurosci.*, **14**, 5302-5324.

Fritschy, J.M., Weinmann, O., Wenzel, A. & Benke, D. (1998) Synapse-specific localization of NMDA- and $GABA_A$-receptor subunits revealed by antigen-retrieval immunohistochemistry. *J. Comp. Neurol.*, **390**, 194-210.

Fuchs, C., Abitbol, K., Burden, J., Mercer, A., Brown, L., Iball, J., Stephenson, A., Thomson, A. & Jovanovic, J. (2013) $GABA_A$ receptors can initiate the formation of functional inhibitory GABAergic synapses. *Eur J Neurosci*, **38**, 3146-3158.

Futai, K., Doty, C., Baek, B., Ryu, J. & Sheng, M. (2013) Specific trans-synaptic interaction with inhibitory interneuronal neurexin underlies differential ability of neuroligins to induce functional inhibitory synapses. *J Neurosci*, **33**, 3612-3623.

Galanopoulou, A. (2010) Mutations affecting GABAergic signaling in seizures and epilepsy. *Pflugers Arch*, **460**, 505-523.

Ge, S., Goh, E.L., Sailor, K.A., Kitabatake, Y., Ming, G.L. & Song, H. (2006) GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature*, **439**, 589-593.

Ge, S., Pradhan, D.A., Ming, G.L. & Song, H. (2007) GABA sets the tempo for activity-dependent adult neurogenesis. *Trends Neurosci.*, **30**, 1-8.

Giannone, G., Mondin, M., Grillo-Bosch, D., Tessier, B., Saint-Michel, E., Czöndör, K., Sainlos, M., Choquet, D. & Thoumine, O. (2013) Neurexin-1β binding to Neuroligin-1 triggers the preferential recruitment of PSD-95 versus gephyrin through tyrosine phosphorylation of Neuroligin-1. *Cell Rep*, **3**, 1996-2007.

Grabenstatter, H., Russek, S. & Brooks-Kayal, A. (2012) Molecular pathways controlling inhibitory receptor expression. *Epilepsia*, **53 Suppl 9**, 71-78.

Graf, E.R., Zhang, X., Jin, S.X., Linhoff, M.W. & Craig, A.M. (2004) Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. *Cell*, **119**, 1013-1026.

Grasshoff, C., Jurd, R., Rudolph, U. & Antkowiak, B. (2007) Modulation of presynaptic β3-containing $GABA_A$ receptors limits the immobilizing actions of GABAergic anesthetics. *Mol. Pharmacol.*, **72**, 780-787.

Gunn, B.G., Brown, A.R., Lambert, J.J. & Belelli, D. (2011) Neurosteroids and $GABA_A$ receptor interactions: A focus on stress. *Frontiers in Neuroscience*, **5**, 131.

Hashimoto, T., Nguyen, Q., Rotaru, D., Keenan, T., Arion, D., Beneyto, M., Gonzalez-Burgos, G. & Lewis, D. (2009) Protracted developmental trajectories of $GABA_A$ receptor α1 and α2 subunit expression in primate prefrontal cortex. *Biol Psychiatry*, **65**, 1015-1023.

Heinrich, C., Lähteinen, S., Suzuki, F., Anne-Marie, L., Huber, S., Häussler, U., Haas, C.A., Larmet, Y., Castren, E. & Depaulis, A. (2011) Increase in BDNF-mediated TrkB signaling promotes epileptogenesis in a mouse model of mesial temporal lobe epilepsy. *Neurobiol. Dis.*, **42**, 35-47.

Heller, E.A., Zhang, W., Selimi, F., Earnheart, J., Ślimak, M.A., Santos-Torres, J., Ibañez-Tallon, I., Aoki, C., Chait, B.T. & Heintz, N. (2012) The biochemical anatomy of cortical inhibitory synapses. *PLoS ONE*, **7**, e39572.

Hennou, S., Khalilov, I., Diabria, D., Ben-Ari, Y. & Gozlan, H. (2002) Early sequential formation of functional $GABA_A$ and glutamatergic synapses on CA1 interneurons of the rat foetal hippocampus. *Eur. J. Neurosci.*, **16**, 197-208.

Hensch, T.K. (2005) Critical period plasticity in local cortical circuits. *Nature Rev. Neurosci.*, **6**, 877-888.

Hensch, T.K., Fagiolini, M., Mataga, N., Stryker, M.P., Baekkeskov, S. & Kash, S.F. (1998) Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science*, **282**, 1504-1508.

Hines, R.M., Davies, P.A., Moss, S.J. & Maguire, J. (2011) Functional regulation of $GABA_A$ receptors in nervous system pathologies. *Cur. Opin. Neurobiol.*, **22**, 552-558.

Hong, E.J., McCord, A.E. & Greenberg, M.E. (2008) A biological function for the neuronal activity-dependent component of BDNF transcription in the development of cortical inhibition. *Neuron*, **60**, 610-624.

Hoon, M., Soykan, T., Falkenburger, B.H., Hammer, M., Patrizi, A., Schmidt, K.F., Sassoè-Pognetto, M., Löwel, S., Moser, T., Taschenberger, H., Brose, N. & Varoqueaux, F. (2011) Neuroligin-4 is localized to glycinergic postsynapses and regulates inhibition in the retina. *Proc. Natl. Acad. Sci. USA*, **108**, 3053-3058.

Hörtnagl, H., Tasan, R., Wieselthaler, A., Kirchmair, E., Sieghart, W. & Sperk, G. (2013) Patterns of mRNA and protein expression for 12 $GABA_A$ receptor subunits in the mouse brain. *Neuroscience*, **236**, 345-372.

Hosie, A.M., Wilkins, M.E., da Silva, H.M. & Smart, T.G. (2006) Endogenous neurosteroids regulate $GABA_A$ receptors through two discrete transmembrane sites. *Nature*, **444**, 486-489.

Houser, C., Zhang, N. & Peng, Z. (2012) Alterations in the Distribution of $GABA_A$ Receptors in Epilepsy. In Noebels, J., Avoli, M., Rogawski, M., Olsen, R., Delgado-Escueta, A. (eds) *Jasper's Basic Mechanisms of the Epilepsies* National Center for Biotechnology Information (US), Bethesda (MD).

Houston, C.M., He, Q. & Smart, T.G. (2009) CaMKII phosphorylation of the $GABA_A$ receptor: receptor subtype- and synapse-specific modulation. *J. Physiol.*, **587**, 2115-2125.

Houston, C.M., Hosie, A.M. & Smart, T.G. (2008) Distinct regulation of β2 and β3 subunit-containing cerebellar synaptic $GABA_A$ receptors by calcium/calmodulin-dependent protein kinase II. *J. Neurosci.*, **28**, 7574-7584.

Husi, H. & Grant, S. (2001) Isolation of 2000-kDa complexes of N-methyl-d-aspartate receptor and postsynaptic density 95 from mouse brain. *J. Neurochem.*, **77**, 281-291.

Hutcheon, B., Morley, P. & Poulter, M.O. (2000) Developmental change in $GABA_A$ receptor desensitization kinetics and its role in synapse function in rat cortical neurons. *J. Physiol.*, **522**, 3-17.

Ibañez-Tallon, I., Miwa, J., Wang, H., Adams, N., Crabtree, G., Sine, S. & Heintz, N. (2002) Novel modulation of neuronal nicotinic acetylcholine receptors by association with the endogenous prototoxin lynx1. *Neuron*, **33**, 893-903.

Jacob, T.C., Moss, S.J. & Jurd, R. (2008) $GABA_A$ receptor trafficking and its role in the dynamic modulation of neuronal inhibition. *Nature Rev. Neurosci.*, **9**, 331-343.

Jagasia, R., Steib, K., Englberger, E., Herold, S., Faus-Kessler, T., Saxe, M., Gage, F.H., Song, H. & Lie, D.C. (2009) GABA-cAMP response element-binding protein signaling regulates maturation and survival of newly generated neurons in the adult hippocampus. *J. Neurosci.*, **24**, 7966-7977.

Jedlicka, P., Hoon, M., Papadopoulos, T., Vlachos, A., Winkels, R., Poulopoulos, A., Betz, H., Deller, T., Brose, N., Varoqueaux, F. & Schwarzacher, S.W. (2011) Increased dentate gyrus excitability in neuroligin-2-deficient mice in vivo. *Cereb. Cortex.*, **21**, 357-367.

Jones, B. & Henderson, L. (2013) Trafficking and potential assembly patterns of ε-containing $GABA_A$ receptors. *J Neurochem*, **103**, 1258-1271.

Jovanovic, J.N., Thomas, P., Kittler, J.T., Smart, T.G. & Moss, S.J. (2004) Brain-derived neurotrophic factor modulates fast synaptic inhibition by regulating $GABA_A$ receptor phosphorylation, activity, and cell-surface stability. *J. Neurosci.*, **24**, 522-530.

Joyce, C. (2007) In silico comparative genomic analysis of $GABA_A$ receptor transcriptional regulation. *BMC Genomics*, **8**, 203.

Jurd, R., Arras, M., Lambert, S., Drexler, B., Siegwart, R., Crestani, F., Zaugg, M., Vogt, K.E., Ledermann, B., Antkowiak, B. & Rudolph, U. (2003) General anesthetic actions in vivo strongly attenuated by a point mutation in the $GABA_A$ receptor β3 subunit. *J. FASEB*, **17**, 250-252.

Jurd, R. & Moss, S. (2010) Impaired $GABA_A$ receptor endocytosis and its correlation to spatial memory deficits. *Commun Integr Biol*, **3**, 176-178.

Kaila, K. (1994) Ionic basis of $GABA_A$ receptor channel function in the nervous system. *Prog. Neurobiol.*, **42**, 489-537.

Kang, Y., Zhang, X., Dobie, F., Wu, H. & Craig, A.M. (2008) Induction of GABAergic Postsynaptic Differentiation by α-Neurexins. *J. Biol. Chem.*, **283**, 2323-2334.

Kano, M., Fukunaga, K. & Kornnerth, A. (1996) Ca^{2+}-induced rebound potentiation of γ-aminobutyric acid-mediated currents requires activation of Ca^{2+}/calmodulin-dependent kinase II. *Proc. Natl. Acad. Sci. USA*, **93**, 13351-13356.

Kasugai, Y., Swinny, J.D., Roberts, J.D., Dalezios, Y., Fukazawa, Y., Sieghart, W., Shigemoto, R. & Somogyi, P. (2010) Quantitative localisation of synaptic and extrasynaptic $GABA_A$ receptor subunits on hippocampal pyramidal cells by freeze-fracture replica immunolabelling. *Eur. J. Neurosci.*, **32**, 1868-1888.

Katagiri, H., Fagiolini, M. & Hensch, T.K. (2007) Optimization of somatic inhibition at critical period onset in mouse visual cortex. *Neuron*, **53**, 805-812.

Kawaguchi, S.Y. & Hirano, T. (2007) Sustained structural change of $GABA_A$ receptor-associated protein underlies long-term potentiation at inhibitory synapses on a cerebellar Purkinje neuron. *J. Neurosci.*, **27**, 6788-6799.

Kennedy, M. (2000) Signal-processing machines at the postsynaptic density. *Science*, **290**, 750-754.

Kins, S., Betz, H. & Kirsch, J. (2000) Collybistin, a newly identified brain-specific GEF, induces submembrane clustering of gephyrin. *Nature Neurosci.*, **3**, 22-29.

Kirsch, J., Wolters, I., Triller, A. & Betz, H. (1993) Gephyrin antisense oligonucleotides prevent glycine receptor clustering in spinal neurons. *Nature*, **366**, 745-748.

Klausberger, T. & Somogyi, P. (2008) Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science*, **321**, 53-57.

Knabl, J., Witschi, R., Hösl, K., Reinold, H., Zeilhofer, U., Ahmadi, S., Brockhaus, J., Sergejeva, M., Hess, A., Brune, K., Fritschy, J., Rudolph, U., Möhler, H. & Zeilhofer, H. (2008) Reversal of pathological pain through specific spinal $GABA_A$ receptor subtypes. *Nature*, **451**, 330-334.

Kneussel, M., Brandstätter, J.H., Gasnier, B., Feng, G., Sanes, J.R. & Betz, H. (2001) Gephyrin-independent clustering of postsynaptic $GABA_A$ receptor subtypes. *Mol. Cell. Neurosci.*, **17**, 973-982.

Koester, C., Rudolph, U., Haenggi, T., Papilloud, A., Fritschy, J. & Crestani, F. (2013) Dissecting the role of diazepam-sensitive γ-aminobutyric acid type A receptors in defensive behavioral reactivity to mild threat. *Pharmacol Biochem Behav*, **103**, 541-549.

Koksma, J.J., Fritschy, J.M., Mack, V., Van Kesteren, R.E. & Brussaard, A.B. (2005) Differential $GABA_A$ receptor clustering determines GABA synapse plasticity in rat oxytocin neurons around parturition and the onset of lactation. *Mol. Cell. Neurosci.*, **28**, 128-140.

Koksma, J.J., Van Kesteren, R.E., Rosahl, T.W., Zwart, R., Smit, A.B., Lüddens, H. & Brussaard, A.B. (2003) Oxytoxin regulates neurosteroid modulation of $GABA_A$ receptors in supraoptic nucleus around parturition. *J. Neurosci.*, **23**, 788-797.

Kowalczyk, S., Winkelmann, A., Smolinsky, B., Förstera, B., Neundorf, I., Schwarz, G. & Meier, J.C. (2013) Direct binding of $GABA_A$ receptor β2 and β3 subunits to gephyrin. *Eur. J. Neurosci.*, **37**, 544-554.

Kralic, J.E., Sidler, C., Parpan, F., Homanics, G., Morrow, A.L. & Fritschy, J.M. (2006) Compensatory alteration of inhibitory synaptic circuits in thalamus and cerebellum of $GABA_A$ receptor α1 subunit knockout mice. *J. Comp. Neurol.*, **495**, 408-421.

Kullmann, D., Moreau, A., Bakiri, Y. & Nicholson, E. (2012) Plasticity of inhibition. *Neuron*, **75**, 951-962.

Laurie, D.J., Seeburg, P.H. & Wisden, W. (1992) The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum. *J. Neurosci.*, **12**, 1063-1076.

Lee, H., Deeb, T., Walker, J., Davies, P. & Moss, S. (2011) NMDA receptor activity downregulates KCC2 resulting in depolarizing $GABA_A$ receptor-mediated currents. *Nat Neurosci*, **14**, 736-743.

Levi, S., Grady, M., Henry, M., Campbell, K., Sanes, J. & Craig, A. (2002) Dystroglycan is selectively associated with inhibitory GABAergic synapses but is dispensable for their differentiation. *J. Neurosci.*, **22**, 4274-4285.

Lewis, D.A. (2012) Cortical circuit dysfunction and cognitive deficits in schizophrenia--implications for preemptive interventions. *Eur J Neurosci.*, **35**, 1871-1878.

Lewis, D.A. & Hashimoto, T. (2007) Deciphering the disease process of schizophrenia: the contribution of cortical gaba neurons. *Int. Rev. Neurobiol.*, **78**, 109-131.

Lewis, D.A., Hashimoto, T. & Volk, D.W. (2005) Cortical inhibitory neurons and schizophrenia. *Nature Rev. Neurosci.*, **6**, 312-324.

Loebrich, S., Bahring, R., Katsuno, T., Tsukita, S. & Kneussel, M. (2006) Activated radixin is essential for $GABA_A$ receptor α5 subunit anchoring at the actin cytoskeleton. *EMBO J.*, **25**, 987-999.

Long, P., Mercer, A., Begum, R., Stephens, G.J., Sihra, T.S. & Jovanovic, J.N. (2009) Nerve terminal $GABA_A$ receptors activate Ca^{2+}/calmodulin-dependent signaling to inhibit voltage-gated Ca^{2+} influx and glutamate release. *J. Biol. Chem.*, **284**, 8726-8737.

Lorincz, A. & Nusser, Z. (2008) Specificity of immunoreactions: the importance of testing specificity in each method. *J. Neurosci.*, **28**, 9083-9086.

Löw, K., Crestani, F., Keist, R., Benke, D., Brünig, I., Benson, J.A., Fritschy, J.M., Rulicke, T., Bluethmann, H., Mohler, H. & Rudolph, U. (2000) Molecular and neuronal substrate for the selective attenuation of anxiety. *Science*, **290**, 131-134.

Lu, J., Hsiao, Y., Chiang, C. & Wang, C. (2013) $GABA_A$ Receptor-Mediated Tonic Depolarization in Developing Neural Circuits. *Mol Neurobiol*, **epub ahead of print**.

Luscher, B., Fuchs, T. & Kilpatrick, C.L. (2011a) $GABA_A$ receptor trafficking-mediated plasticity of inhibitory synapses. *Neuron*, **70**, 385-409.

Luscher, B., Shen, Q. & Sahir, N. (2011b) The GABAergic deficit hypothesis of major depressive disorder. *Mol Psychiatry*, **16**, 383-406.

Macdonald, R., Kang, J. & Gallagher, M. (2010) Mutations in $GABA_A$ receptor subunits associated with genetic epilepsies. *J Physiol.*, **588**, 1861-1869.

Maguire, J. & Mody, I. (2009) Steroid hormone fluctuations and $GABA_AR$ plasticity. *Psychoneuroendocrinol.*, **34**, S84-90.

Mann, E.O. & Mody, I. (2010) Control of hippocampal gamma oscillation frequency by tonic inhibition and excitation of interneurons. *Nature Neurosci.*, **13**, 205-212.

Marín, O. (2012) Interneuron dysfunction in psychiatric disorders. *Nature Rev Neurosci*, **13**, 107-120.

Marowsky, A., Rudolph, U., Fritschy, J.M. & Arand, M. (2012) Tonic inhibition in principal cells of the amygdala: A central role for α3 subunit-containing $GABA_A$ receptors. *J. Neurosci.*, **32**, 8611-8619.

Martínez-Cué, C., Martínez, P., Rueda, N., Vidal, R., García, S., Vidal, V., Corrales, A., Montero, J., Pazos, Á., Flórez, J., Gasser, R., Thomas, A., Honer, M., Knoflach, F., Trejo, J., Wettstein, J. & Hernández, M. (2013) Reducing $GABA_A$ α5 receptor-mediated inhibition rescues functional and neuromorphological deficits in a mouse model of Down syndrome. *J Neurosci*, **33**, 3953-3966.

Masneuf, S., Buetler, J., Koester, C. & Crestani, F. (2012) Role of α1- and α2-$GABA_A$ receptors in mediating the respiratory changes associated with benzodiazepine sedation. *Br J Pharmacol*, **166**, 339-348.

Mayer, S., Kumar, R., Jaiswal, M., Soykan, T., Ahmadian, M., Brose, N., Betz, H., Rhee, J. & Papadopoulos, T. (2013) Collybistin activation by GTP-TC10 enhances postsynaptic gephyrin clustering and hippocampal GABAergic neurotransmission. *Proc Natl Acad Sci USA*, **110**, 20795-20800.

Merz, K., Herold, S. & Lie, D.C. (2011) CREB in adult neurogenesis--master and partner in the development of adult-born neurons? *Eur. J. Neurosci.*, **33**, 1078-1086.

Milenkovic, I., Vasiljevic, M., Maurer, D., Höger, H., Klausberger, T. & Sieghart, W. (2013) The parvalbumin-positive interneurons in the mouse dentate gyrus express $GABA_A$ receptor subunits α1, β2, and δ along their extrasynaptic cell membrane. *Neuroscience*, **254C**, 80-96.

Morishita, H., Miwa, J., Heintz, N. & Hensch, T. (2010) Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. *Science*, **330**, 1238-1240.

Mortensen, M., Ebert, B., Wafford, K. & Smart, T.G. (2010) Distinct activities of GABA agonists at synaptic- and extrasynaptic-type $GABA_A$ receptors. *J. Physiol. (Lond.)*, **588**, 1251-1268.

Mou, L., Dias, B., Gosnell, H. & Ressler, K. (2013) Gephyrin plays a key role in BDNF-dependent regulation of amygdala surface $GABA_A$Rs. *Neuroscience*, **Epub ahead of print**.

Muir, J., Arancibia-Carcamo, I., MacAskill, A., Smith, K., Griffin, L. & Kittler, J. (2010) NMDA receptors regulate $GABA_A$ receptor lateral mobility and clustering at inhibitory synapses through serine 327 on the γ2 subunit. *Proc Natl Acad Sci USA*, **107**, 16679-16684.

Mukherjee, J., Kretschmannova, K., Gouzer, G., Maric, H.M., Ramsden, S.L., Tretter, V., Harvey, K., Davies, P.A., Triller, A., Schindelin, H. & Moss, S.J. (2011) The residence time of $GABA_A$Rs at inhibitory synapses is determined by direct binding of the receptor α1 subunit to gephyrin. *J. Neurosci.*, **31**, 14677-14687.

Mulligan, M., Wang, X., Adler, A., Mozhui, K., Lu, L. & Williams, R. (2012) Complex control of $GABA_A$ receptor subunit mRNA expression: variation, covariation, and genetic regulation. *PLoS ONE*, **7**, e34586.

Nani, F., Bright, D., Revilla-Sanchez, R., Tretter, V., Moss, S. & Smart, T. (2013) Tyrosine phosphorylation of $GABA_A$ receptor γ2-subunit regulates tonic and phasic inhibition in the thalamus. *J Neurosci*, **33**, 12718-12727.

Nissant, A. & Pallotto, M. (2011) Integration and maturation of newborn neurons in the adult olfactory bulb--from synapses to function. *Eur. J. Neurosci.*, **33**, 1069-1077.

Niwa, F., Bannai, H., Arizono, M., Fukatsu, K., Triller, A. & Mikoshiba, K. (2012) Gephyrin-independent $GABA_AR$ mobility and clustering during plasticity. *PLoS ONE*, **7**, e36148.

Notter, T., Panzanelli, P., Pfister, S., Mircsof, D. & Fritschy, J. (2013) A protocol for concurrent high-quality immunohistochemical and biochemical analyses in adult mouse central nervous system. *Eur J Neurosci*, **[Epub ahead of print]**.

Nusser, Z., Ahmad, Z., Tretter, V., Fuchs, K., Wisden, W., Sieghart, W. & Somogyi, P. (1999) Alterations in the expression of $GABA_A$ receptor subunits in cerebellar granule cells after the disruption of the α6 subunit gene. *Eur. J. Neurosci.*, **11**, 1685-1697.

Nusser, Z., Roberts, J.D.B., Baude, A., Richards, J.G., Sieghart, W. & Somogyi, P. (1995) Immunocytochemical localization of the α1 and β2/3 subunits of the $GABA_A$ receptor in relation to specific GABAergic synapses in the dentate gyrus. *Eur. J. Neurosci.*, **7**, 630-646.

Nusser, Z., Sieghart, W., Benke, D., Fritschy, J.M. & Somogyi, P. (1996) Differential synaptic localization of two major γ-aminobutyric acid type A receptor α subunits on hippocampal pyramidal neurons. *Proc. Natl. Acad. Sci. USA*, **93**, 11939-11944.

Nusser, Z., Sieghart, W. & Somogyi, P. (1998) Segregation of different $GABA_A$ receptors to synaptic and extrasynaptic membranes of cerebellar granule cells. *J. Neurosci.*, **18**, 1693-1703.

Ogris, W., Lehner, R., Fuchs, K., Furtmuller, B., Hoger, H., Homanics, G.E. & Sieghart, W. (2006) Investigation of the abundance and subunit composition of $GABA_A$ receptor subtypes in the cerebellum of α1-subunit-deficient mice. *J. Neurochem.*, **96**, 136-147.

Olsen, R.W. & Sieghart, W. (2008) International Union of Pharmacology. LXX. Subtypes of γ-aminobutyric $acid_A$ receptors: classification on the basis of subunit composition, pharmacology, and function. Update. *Pharmacol Rev*, **60**, 243-260.

Overstreet, W.L., Bromberg, D.A., Benson, A.L. & Westbrook, G.L. (2005) GABAergic signaling to newborn neuron in dentate gyrus. *J. Neurophysiol.*, **94**, 4528-4532.

Pallotto, M., Nissant, A., Fritschy, J.M., Rudolph, U., Sassoè-Pognetto, M., Panzanelli, P. & Lledo, P.M. (2012) Early formation of GABAergic synapses governs the development of adult-born neurons in the olfactory bulb. *J. Neurosci.*, **32**, 9103-9115.

Panzanelli, P., Gunn, B.G., Schlatter, M.C., Benke, D., Tyagarajan, S.K., Scheiffele, P., Belelli, D., Lambert, J.J., Rudolph, U. & Fritschy, J.M. (2011) Distinct mechanisms regulate $GABA_A$ receptor and gephyrin clustering at perisomatic and axo-axonic synapses on CA1 pyramidal cells. *J. Physiol.*, **589**, 4959-4980.

Panzanelli, P., Homanics, G.E., Ottersen, O.P., Fritschy, J.M. & Sassoè-Pognetto, M. (2004) Pre- and postsynaptic $GABA_A$ receptors at reciprocal dendrodendritic synapses in the olfactory bulb. *Eur. J. Neurosci.*, **20**, 2945-2952.

Papadopoulos, T., Eulenburg, V., Reddy-Alla, S., Mansuy, I.M., Li, Y. & Betz, H. (2008) Collybistin is required for both the formation and maintenance of GABAergic postsynapses in the hippocampus. *Mol. Cell. Neurosci.*, **39**, 161-169.

Papadopoulos, T., Korte, M., Eulenburg, V., Kubota, H., Retiounskaia, M., Harvey, R.J., Harvey, K., O'sullivan, G.A., Laube, B., Hulsmann, S., Geiger, J.R. & Betz, H. (2007) Impaired GABAergic transmission and altered hippocampal synaptic plasticity in collybistin-deficient mice. *EMBO J.*, **26**, 3888-3899.

Passlick, S., Grauer, M., Schäfer, C., Jabs, R., Seifert, G. & Steinhäuser, C. (2013) Expression of the γ2-subunit distinguishes synaptic and extrasynaptic $GABA_A$ receptors in NG2 cells of the hippocampus. *J Neurosci*, **33**, 12030-12040.

Patel, B., Mortensen, M. & Smart, T. (2013) Stoichiometry of δ subunit containing $GABA_A$ receptors. *Br J Pharmacol*, **Epub ahead of print**.

Paul, J., Yévenes, G., Benke, D., Lio, A., Ralvenius, W., Witschi, R., Scheurer, L., Cook, J., Rudolph, U., Fritschy, J. & Zeilhofer, H. (2013) Antihyperalgesia by α2-$GABA_A$ Receptors Occurs Via a Genuine Spinal Action and Does Not Involve Supraspinal Sites. *Neuropsychopharmacol.*, **Epub ahead of print**.

Paul, J., Zeilhofer, H.U. & Fritschy, J.M. (2012) Selective distribution of $GABA_A$ receptor subtypes in mouse spinal dorsal horn neurons and primary afferents. *J. Comp. Neurol.*, **520**, 3895-3911.

Pavlov, I., Kaila, K., Kullmann, D. & Miles, R. (2013) Cortical inhibition, pH and cell excitability in epilepsy: what are optimal targets for antiepileptic interventions? *J Physiol.*, **591**, 765-774.

Paysan, J., Bolz, J., Mohler, H. & Fritschy, J.M. (1994) The $GABA_A$-receptor α1-subunit: an early marker of cortical parcellation. *J. Comp. Neurol.*, **350**, 133-149.

Paysan, J., Kossel, A., Bolz, J. & Fritschy, J.M. (1997) Area-specific regulation of γ-aminobutyric acid A receptor subtypes by thalamic afferents in developing rat neocortex. *Proc. Natl. Acad. Sci. USA*, **94**, 6995-7000.

Peden, D.R., Petitjean, C.M., Herd, M.B., Durakoglugil, M., Rosahl, T.W., Wafford, K., Homanics, G.E., Belelli, D., Fritschy, J.M. & Lambert, J.J. (2008) Developmental maturation of synaptic

and extrasynaptic $GABA_A$ receptors in mouse thalamic ventrobasal neurones. *J. Physiol.*, **586**, 965-987.

Peixoto, R.T., Kunz, P.A., Kwon, H., Mabb, A.M., Sabatini, B.L., Philpot, B.D. & Ehlers, M.D. (2012) Transsynaptic signaling by activity-dependent cleavage of neuroligin-1. *Neuron*, **76**, 396-409.

Peng, Z., Hauer, B., Mihalek, R.M., Homanics, G.E., Sieghart, W., Olsen, R.W. & Houser, C.R. (2002) $GABA_A$ receptor changes in δ subunit-deficient mice: Altered expression of α4 and γ2 subunits in the forebrain. *J. Comp. Neurol.*, **446**, 179-197.

Pfeffer, C., Xue, M., He, M., Huang, Z. & Scanziani, M. (2013) Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. *Nat Neurosci*, **16**, 1068-1076.

Pfeiffer, F., Graham, D. & H., B. (1982) Purification by affinity chromatography of the glycine receptor of rat spinal cord. *J Biol Chem*, **257**, 9389-9393.

Pizzarelli, R. & Cherubini, E. (2011) Alterations of GABAergic signaling in autism spectrum disorders. *Neural Plast*, 2011:297153.

Platel, J.C., Lacar, B. & Bordey, A. (2007) GABA and glutamate signaling: homeostatic control of adult forebrain neurogenesis. *J Mol Histol.*, **38**, 602-610.

Polan, M., Pastore, M., Steingass, K., Hashimoto, S., Thrush, D., Pyatt, R., Reshmi, S., Gastier-Foster, J., Astbury, C. & McBride, K. (2013) Neurodevelopmental disorders among individuals with duplication of 4p13 to 4p12 containing a $GABA_A$ receptor subunit gene cluster. *Eur J Hum Genet*, **Epub ahead of print**.

Poulopoulos, A., Aramuni, G., Meyer, G., Soykan, T., Hoon, M., Papadopoulos, T., Zhang, M., Paarmann, I., Fuchs, C., Harvey, K., Jedlicka, P., Schwarzacher, S.W., Betz, H., Harvey, R.J., Brose, N., Zhang, W. & Varoqueaux, F. (2009) Neuroligin 2 drives postsynaptic assembly at perisomatic inhibitory synapses through gephyrin and collybistin. *Neuron*, **63**, 628-642.

Poulopoulos, A., Soykan, T., Tuffy, L., Hammer, M., Varoqueaux, F. & Brose, N. (2012) Homodimerization and isoform-specific heterodimerization of neuroligins. *Biochem J*, **446**, 321-330.

Prut, L., Prenosil, G.A., Willadt, S., Vogt, K., Fritschy, J.M. & Crestani, F. (2010) A reduction in hippocampal $GABA_A$ receptor α5 subunits disrupts the memory for location of objects in mice. *Genes Brain Behav*, **9**, 478-488.

Puskarjov, M., Ahmad, F., Kaila, K. & Blaesse, P. (2012) Activity-dependent cleavage of the K-Cl cotransporter KCC2 mediated by calcium-activated protease calpain. *J Neurosci.*, **32**, 11356-11364.

Raimondo, J., Markram, H. & Akerman, C. (2012) Short-term ionic plasticity at GABAergic synapses. *Front Synaptic Neurosci*, **4**, 5.

Reddy-Alla, S., B., S., Birkenfeld, J., Eulenburg, V., Dutertre, S., Böhringer, C., Götz, M., Betz, H. & Papadopoulos, T. (2010) PH-Domain-driven targeting of collybistin but not Cdc42 activation is required for synaptic gephyrin clustering. *Eur. J. Neurosci.*, **31**, 1173-1184.

Renner, M., Schweizer, C., Bannai, H., Triller, A. & Lévi, S. (2012) Diffusion barriers constrain receptors at synapses. *PLoS ONE*, **7**, e43032.

Represa, A. & Ben-Ari, Y. (2005) Trophic actions of GABA on neuronal development. *Trends Neurosci*, **28**, 278-283.

Rivera, C., Voipio, J. & Kaila, K. (2005) Two developmental switches in GABAergic signalling: the K^+Cl^- cotransporter KCC2 and carbonic anhydrase CAVII. *J. Physiol.*, **562**, 27-36.

Rudolph, U. & Antkowiak, B. (2004) Molecular and neuronal substrates for general anaesthetics. *Nature Rev. Neurosci.*, **5**, 709-720.

Rudolph, U., Crestani, F., Benke, D., Brünig, I., Benson, J., Fritschy, J.M., Martin, J.R., Bluethmann, H. & Mohler, H. (1999) Benzodiazepine actions mediated by specific γ-aminobutyric $acid_A$ receptor subtypes. *Nature*, **401**, 796-800.

Rudolph, U. & Möhler, H. (2004) Analysis of $GABA_A$ receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. *Annu. Rev. Pharmacol. Toxicol.*, **44**, 475-498.

Rudolph, U. & Möhler, H. (2013) $GABA_A$ receptor subtypes: Therapeutic potential in Down syndrome, affective disorders, schizophrenia, and autism. *Annu Rev Pharmacol Toxicol*, **Epub ahead of print**.

Rui, Y., Myers, K., Yu, K., Wise, A., De Blas, A., Hartzell, H. & Zheng, J. (2013) Activity-dependent regulation of dendritic growth and maintenance by glycogen synthase kinase 3β. *Nat Commun*, **4**, 2628.

Ruiz, A., Campanac, E., Scott, R., Rusakov, D. & Kullmann, D. (2010) Presynaptic $GABA_A$ receptors enhance transmission and LTP induction at hippocampal mossy fiber synapses. *Nature Neurosci*, **13**, 431-438.

Ruusuvuori, E., Huebner, A., Kirilkin, I., Yukin, A., Blaesse, P., Helmy, M., Kang, H., El Muayed, M., Hennings, J., Voipio, J., Šestan, N., Hübner, C. & Kaila, K. (2013) Neuronal carbonic anhydrase VII provides GABAergic excitatory drive to exacerbate febrile seizures. *EMBO J*, **32**, 2275-2286.

Saghatelyan, A., Roux, P., Migliore, M., Rochefort, C., Desmaisons, D., Charneau, P., Shepherd, G.M. & Lledo, P.M. (2005) Activity-dependent adjustments of the inhibitory network in the olfactory bulb following early postnatal deprivation. *Neuron*, **46**, 103-116.

Saiepour, L., Fuchs, C., Patrizi, A., Sassoè-Pognetto, M., Harvey, R.J. & Harvey, K. (2010) Complex role of collybistin and gephyrin in $GABA_A$ receptor clustering. *J. Biol. Chem.*, **285**, 29623-29631.

Sander, B., Tria, G., Shkumatov, A., Kim, E., Grossmann, J., Tessmer, I., Svergun, D. & Schindelin, H. (2013) Structural characterization of gephyrin by AFM and SAXS reveals a mixture of compact and extended states. *Acta Crystallogr D Biol Crystallogr*, **69**, 2050-2060.

Sarkar, J., Wakefield, S., MacKenzie, G., Moss, S.J. & Maguire, J. (2011) Neurosteroidogenesis is required for the physiological response to stress: role of neurosteroid-sensitive $GABA_A$ receptors. *J. Neurosci.*, **31**, 18198-18210.

Sassoè-Pognetto, M., Kirsch, J., Grünert, U., Greferath, U., Fritschy, J.M., Mohler, H., Betz, H. & Wässle, H. (1995) Colocalization of gephyrin and $GABA_A$-receptor subunits in the rat retina. *J. Comp. Neurol.*, **357**, 1-14.

Sassoè-Pognetto, M., Panzanelli, P., Sieghart, W. & Fritschy, J.M. (2000) Co-localization of multiple $GABA_A$ receptor subtypes with gephyrin at postsynaptic sites. *J. Comp. Neurol.*, **420**, 481-498.

Scheiffele, P., Fan, J., Choih, J., Fetter, R. & Serafini, T. (2000) Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. *Cell*, **101**, 657-669.

Schneider Gasser, E.M., Duveau, V., Prenosil, G.A. & Fritschy, J.M. (2007) Reorganization of GABAergic circuits maintains $GABA_A$ receptor-mediated transmission onto CA1 interneurons in α1−subunit-null mice. *Eur. J. Neurosci.*, **25**, 3287-3304.

Schofield, C.M., Kleiman-Weiner, M., Rudolph, U. & Huguenard, J.R. (2009) A gain in $GABA_A$ receptor synaptic strength in thalamus reduces oscillatory activity and absence seizures. *Proc. Natl. Acad. Sci. USA*, **106**, 7630-7635.

Schwarz, G., Mendel, R.R. & Ribbe, M.W. (2009) Molybdenum cofactors, enzymes and pathways. *Nature*, **460**, 839-847.

Schweizer, C., Balsiger, S., Bluethmann, H., Mansuy, I.M., Fritschy, J.M., Mohler, H. & Luscher, B. (2003) The γ2 subunit of $GABA_A$ receptors is required for maintenance of receptors at mature synapses. *Mol. Cell. Neurosci.*, **24**, 442-450.

Sernagor, E., Chabrol, F., Bony, G. & Cancedda, L. (2010) GABAergic control of neurite outgrowth and remodeling during development and adult neurogenesis: general rules and differences in diverse systems. *Front Cell Neurosci*, **4**, 11.

Serwanski, D.R., Miralles, C.P., Christie, S.B., Mehta, A.K., Li, X. & De Blas, A.L. (2006) Synaptic and nonsynaptic localization of $GABA_A$ receptors containing the α5 subunit in the rat brain. *J. Comp. Neurol.*, **499**, 458-470.

Shen, K. & Scheiffele, P. (2010) Genetics and cell biology of building specific synaptic connectivity. *Annu Rev Neurosci*, **33**, 473-507.

Shen, Q., Fuchs, T., Sahir, N. & Luscher, B. (2012) GABAergic control of critical developmental periods for anxiety- and depression-related behavior in mice. *PLoS ONE*, **7**, e47441.

Sheng, M. & Sala, C. (2001) PDZ domains and the organization of supramolecular complexes. *Annu. Rev. Neurosci.*, **24**, 1-29.

Shipman, S.L. & Nicoll, R.A. (2012) Dimerization of postsynaptic neuroligin drives synaptic assembly via transsynaptic clustering of neurexin. *Proc Natl Acad Sci USA*, **109**, 19432-19437.

Shrivastava, A.N., Triller, A., Sieghart, W. & Sarto-Jackson, I. (2011) Regulation of $GABA_A$ receptor dynamics by interaction with purinergic P2X(2) receptors. *J. Biol. Chem.*, **286**, 14455-14468.

Simon, J., Wakimoto, H., Fujita, N., Lalande, M. & Barnard, E. (2004) Analysis of the set of $GABA_A$ receptor genes in the human genome. *J Biol Chem*, **279**, 41422-41435.

Smith, K., Muir, J., Rao, Y., Browarski, M., Gruenig, M., Sheehan, D., Haucke, V. & Kittler, J. (2012) Stabilization of $GABA_A$ receptors at endocytic zones is mediated by an AP2 binding motif within the $GABA_A$ receptor β3 subunit. *J Neurosci*, **32**, 2485-2498.

Smith, S. (2013) The influence of stress at puberty on mood and learning: role of the αβδ $GABA_A$ receptor. *Neuroscience*, **249**, 192-213.

Sohal, V., Zhang, F., Yizhar, O. & Deisseroth, K. (2009) Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature*, **459**, 698-702.

Somogyi, P., Fritschy, J.M., Benke, D., Roberts, J.D.B. & Sieghart, W. (1996) The γ2 subunit of the $GABA_A$-receptor is concentrated in synaptic junctions containing the α1 and β2/3 subunits in hippocampus, cerebellum and globus pallidus. *Neuropharmacol.*, **35**, 1425-1444.

Somogyi, P., Takagi, H., Richards, J.G. & Mohler, H. (1989) Subcellular localization of benzodiazepine/$GABA_A$ receptors in the cerebellum of rat, cat, and monkey using monoclonal antibodies. *J. Neurosci.*, **9**, 2197-2209.

Song, J., Zhong, C., Bonaguidi, M.A., Sun, G.J., Hsu, D., Gu, Y., Meletis, K., Huang, Z.J., Ge, S., Enikolopov, G., Deisseroth, K., Luscher, B., Christian, K.M., Ming, G.L. & Song, H. (2012) Neuronal circuitry mechanism regulating adult quiescent neural stem-cell fate decision. *Nature*, **489**, 150-154.

Song, J.Y., Ichtchenko, K., Sudhof, T.C. & Brose, N. (1999) Neuroligin 1 is a postsynaptic cell-adhesion molecule of excitatory synapses. *Proc. Natl. Acad. Sci. (USA)*, **96**, 1100-1105.

Soto, F., Bleckert, A., Lewis, R., Kang, Y., Kerschensteiner, D., Craig, A. & Wong, R. (2011) Coordinated increase in inhibitory and excitatory synapses onto retinal ganglion cells during development. *Neural Dev*, **6**, 31.

Specht, C., Izeddin, I., Rodriguez, P., El Beheiry, M., Rostaing, P., Darzacq, X., Dahan, M. & Triller, A. (2013) Quantitative nanoscopy of inhibitory synapses: counting gephyrin molecules and receptor binding sites. *Neuron*, **79**, 308-321.

Stallmeyer, B., Schwarz, G., Schulze, J., Nerlich, A., Reiss, J., Kirsch, J. & Mendel, R.R. (1999) The neurotransmitter receptor-anchoring protein gephyrin reconstitutes molybdenum cofactor biosynthesis in bacteria, plants, and mammalian cells. *Proc. Natl. Acad. Sci. USA*, **96**, 1333-1338.

Steiger, J. & Russek, S. (2004) GABAA receptors: building the bridge between subunit mRNAs, their promoters, and cognate transcription factors. *Pharmacol Ther*, **101**, 259-281.

Studer, R., von Boehmer, L., Haenggi, T., Schweizer, C., Benke, D., Rudolph, U. & Fritschy, J.M. (2006) Alteration of GABAergic synapses and gephyrin clusters in the thalamic reticular nucleus of $GABA_A$ receptor α3 subunit-null mice. *Eur. J. Neurosci.*, **24**, 1307-1315.

Südhof, T.C. (2008) Neuroligins and neurexins link synaptic function to cognitive disease. *Nature*, **455**, 903-911.

Sumita, K., Sato, Y., Iida, J., Kawata, A., Hamano, M., Hirabayashi, S., Ohno, K., Peles, E. & Hata, Y. (2007) Synaptic scaffolding molecule (S-SCAM) membrane-associated guanylate kinase with inverted organization (MAGI)-2 is associated with cell adhesion molecules at inhibitory synapses in rat hippocampal neurons. *J. Neurochem.*, **100**, 154-166.

Sun, C., Cheng, M., Qin, R., Liao, D., Chen, T., Koong, F., Chen, G. & Chen, C. (2011) Identification and functional characterization of rare mutations of the neuroligin-2 gene (NLGN2) associated with schizophrenia. *Hum Mol Genet*, **20**, 3042-3051.

Sun, C., Zhang, L. & Chen, G. (2013) An unexpected role of neuroligin-2 in regulating KCC2 and GABA functional switch. *Mol Brain*, **6**, 23.

Suzuki, K., Hayashi, Y., Nakahara, S., Kumazaki, H., Prox, J., Horiuchi, K., Zeng, M., Tanimura, S., Nishiyama, Y., Osawa, S., Sehara-Fujisawa, A., Saftig, P., Yokoshima, S., Fukuyama, T., Matsuki, N., Koyama, R., Tomita, T. & Iwatsubo, T. (2012) Activity-dependent proteolytic cleavage of neuroligin-1. *Neuron*, **76**, 410-422.

Tan, K., Brown, M., Labouèbe, G., Yvon, C., Creton, C., Fritschy, J., Rudolph, U. & Lüscher, C. (2010) Neural bases for addictive properties of benzodiazepines. *Nature*, **463**, 769-774.

Tanaka, S., Kawaguchi, S., Shioi, G. & Hirano, T. (2013) Long-term Potentiation of Inhibitory Synaptic Transmission onto Cerebellar Purkinje Neurons Contributes to Adaptation of Vestibulo-Ocular Reflex. *J Neurosci*, **33**, 17209-17220.

Tanaka, Y., Tozuka, Y., Takata, T., Shimazu, N., Matsumura, N., Ohta, A. & Hisatsune, T. (2009) Excitatory GABAergic activation of cortical dividing glial cells. *Cereb Cortex*, **19**, 2181-2195.

Tao, W., Higgs, M., Spain, W. & Ransom, C. (2013) Postsynaptic $GABA_B$ receptors enhance extrasynaptic $GABA_A$ receptor function in dentate gyrus granule cells. *J Neurosci*, **33**, 3738-3743.

Thomas, P., Mortensen, M., Hosie, A.M. & Smart, T.G. (2005) Dynamic mobility of functional $GABA_A$ receptors at inhibitory synapses. *Nature Neurosci.*, **8**, 889-897.

Tretter, V., Jacob, T.C., Mukherjee, J., Fritschy, J.M., Pangalos, M.N. & Moss, S.J. (2008) The clustering of $GABA_A$ receptor subtypes at inhibitory synapses is facilitated via the direct binding of receptor α2 subunits to gephyrin. *J. Neurosci.*, **28**, 1356-1365.

Tretter, V., Kerschner, B., Milenkovic, I., Ramsden, S.L., Ramerstorfer, J., Saiepour, L., Maric, H.M., Moss, S.J., Schindelin, H., Harvey, R.J., Sieghart, W. & Harvey, K. (2011) Molecular basis of the $GABA_A$ receptor α3 subunit interaction with gephyrin. *J. Biol. Chem.*, **286**, 42105-42114.

Tretter, V., Mukherjee, J., Maric, H.M., Schindelin, H., Sieghart, W. & Moss, S.J. (2012) Gephyrin, the enigmatic organizer at GABAergic synapses. *Front. Cell. Neurosci.*, **6**, 23.

Trigo, F.F., Marty, A. & Stell, B.M. (2008) Axonal $GABA_A$ receptors. *Eur. J. Neurosci.*, **28**, 841-848.

Triller, A., Cluzeaud, F., Pfeiffer, F., Betz, H. & Korn, H. (1985) Distribution of glycine receptors at central synapses: an immunoelectron microscopy study. *J. Cell Biol.*, **101**, 683-688.

Tukker, J.J., Fuentealba, P., Hartwich, K., Somogyi, P. & Klausberger, T. (2007) Cell type-specific tuning of hippocampal interneuron firing during gamma oscillations in vivo. *J. Neurosci.*, **27**, 8184-8189.

Tyagarajan, S., Ghosh, H., Yévenes, G., Imanishi, S., Zeilhofer, H., Gerrits, B. & Fritschy, J. (2013) Extracellular signal-regulated kinase and glycogen synthase kinase 3β regulate gephyrin postsynaptic aggregation and GABAergic synaptic function in a calpain-dependent mechanism. *J Biol Chem*, **288**, 9634-9647.

Tyagarajan, S.K. & Fritschy, J.M. (2014) Gephyrin, a master regulator of neuronal function? *Nature Rev. Neurosci.*, **in press**.

Tyagarajan, S.K., Ghosh, H., Harvey, K. & Fritschy, J.M. (2011a) Collybistin splice variants differentially interact with gephyrin and Cdc42 to regulate gephyrin clustering at GABAergic synapses. *J. Cell Sci.*, **124**, 2786-2796.

Tyagarajan, S.K., Ghosh, H., Yevenes, G.E., Nikonenko, I., Ebeling, C., Schwerdel, C., Sidler, C., Zeilhofer, H.U., Gerrits, B., Muller, D. & Fritschy, J.M. (2011b) Regulation of GABAergic synapse formation and plasticity by GSK3beta-dependent phosphorylation of gephyrin. *Proc. Natl. Acad. Sci. USA*, **108**, 379-384.

Uusi-Oukari, M., Heikkilä, J., Sinkkonen, S.T., Mäkelä, R., Hauer, B., Homanics, G.E., Sieghart, W., Wisden, W. & Korpi, E.R. (2000) Long-range interactions in neuronal gene expression: evidence from gene targeting in the $GABA_A$ receptor β2-α6-α1-γ2 subunit gene cluster. *Mol. Cell. Neurosci.*, **16**, 34-41.

van Rijnsoever, C., Täuber, M., Choulli, M.K., Keist, R., Rudolph, U., Mohler, H., Frischy, J.M. & Crestani, F. (2004) Requirement of α5-$GABA_A$ receptors for the development of tolerance to the sedative action of diazepam in mice. *J. Neurosci.*, **24**, 6785-6790.

Varoqueaux, F., Aramuni, G., Rawson, R.L., Mohrmann, R., Missler, M., Gottmann, K., Zhang, W., Suedhof, T.C. & Brose, N. (2006) Neuroligins determine synapse maturation and function. *Neuron*, **51**, 741-754.

Varoqueaux, F., Jamain, S. & Brose, N. (2004) Neuroligin 2 is exclusively localized to inhibitory synapses. *Eur. J. Cell Biol.*, **83**, 449-456.

Vicini, S., Ferguson, C., Prybylowski, K., Kralic, J.E., Morrow, A.L. & Homanics, G.E. (2001) $GABA_A$ receptor α1 subunit deletion prevents developmental changes of inhibitory synaptic currents in cerebellar neurons. *J. Neurosci.*, **21**, 3009-3016.

Viitanen, T., Ruusuvuori, E., Kaila, K. & Voipio, J. (2010) The K^+-Cl^- cotransporter KCC2 promotes GABAergic excitation in the mature rat hippocampus. *J Physiol.*, **588**, 1527-1540.

Vithlani, M., Hines, R., Zhong, P., Terunuma, M., Hines, D., Revilla-Sanchez, R., Jurd, R., Haydon, P., Rios, M., Brandon, N., Yan, Z. & Moss, S. (2013) The Ability of BDNF to Modify Neurogenesis and Depressive-Like Behaviors Is Dependent upon Phosphorylation of Tyrosine Residues 365/367 in the $GABA_A$-Receptor γ2 Subunit. *J Neurosci*, **33**, 15567-15577.

Vithlani, M., Terunuma, M. & Moss, S. (2011) The dynamic modulation of $GABA_A$ receptor trafficking and its role in regulating the plasticity of inhibitory synapses. *Physiol Rev*, **91**, 1009-1022.

Wakita, M., Kotani, N., Kogure, K. & Akaike, N. (2013) Inhibition of excitatory synaptic transmission in hippocampal neurons by levetiracetam involves Zn^{2+}-dependent $GABA_A$ receptor-mediated presynaptic modulation. *J Pharmacol Exp Ther*, **Epub ahead of print**.

Wan, Q., Xiong, Z.G., Man, H.Y., Ackerley, C.A., Braunton, J., Lu, W.Y., Becker, L.E., MacDonald, J.F. & Wang, Y.T. (1997) Recruitment of functional $GABA_A$ receptors to postsynaptic domains by insulin. *Nature*, **388**, 686-690.

Wang, D., Zurek, A., Lecker, I., Yu, J., Abramian, A., Avramescu, S., Davies, P., Moss, S., Lu, W. & Orser, B. (2012) Memory deficits induced by inflammation are regulated by α5-subunit-containing $GABA_A$ receptors. *Cell Rep*, **2**, 488-496.

Whissell, P., Rosenzweig, S., Lecker, I., Wang, D., Wojtowicz, J. & Orser, B. (2013) γ-aminobutyric acid type A receptors that contain the δ subunit promote memory and neurogenesis in the dentate gyrus. *Ann Neurol*, **Epub ahead of print**.

Wisden, W., Laurie, D.J., Monyer, H. & Seeburg, P.H. (1992) The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J. Neurosci.*, **12**, 1040-1062.

Witschi, R., Punnakkal, P., Paul, J., Walczak, J.S., Cervero, F., Fritschy, J.M., Kuner, R., Keist, R., Rudolph, U. & Zeilhofer, H.U. (2011) Presynaptic α2-$GABA_A$ receptors in primary afferent depolarization and spinal pain control. *J. Neurosci.*, **31**, 8134-8142.

Wlodarczyk, A., Sylantyev, S., Herd, M., Kersanté, F., Lambert, J., Rusakov, D., Linthorst, A., Semyanov, A., Belelli, D., Pavlov, I. & Walker, M. (2013) GABA-independent $GABA_A$ receptor openings maintain tonic currents. *J Neurosci*, **33**, 3905-3914.

Woo, J., Kwon, S., Nam, J., Choi, S., Takahashi, H., Krueger, D., Park, J., Lee, Y., Bae, J., Lee, D., Ko, J., Kim, H., Kim, M., Bae, Y., Chang, S., Craig, A. & Kim, E. (2013) The adhesion protein IgSF9b is coupled to neuroligin 2 via S-SCAM to promote inhibitory synapse development. *J Cell Biol*, **201**, 929-944.

Wu, X., Wu, Z., Ning, G., Guo, Y., Ali, R., Macdonald, R., De Blas, A., Luscher, B. & Chen, G. (2012) $GABA_A$ receptor α subunits play a direct role in synaptic versus extrasynaptic targeting. *J. Biol. Chem.*, **287**, 27417-27430.

Xu, G., Broadbelt, K.G., Haynes, R.L., Folkerth, R.D., Borenstein, N.S., Belliveau, R.A., Trachtenberg, F.L., Volpe, J.J. & Kinney, H.C. (2011) Late development of the GABAergic system in the human cerebral cortex and white matter. *J. Neurophatol. Exp. Neurol.*, **70**, 841-858.

Yee, B.K., Keist, R., von Boehmer, L., Studer, R., Benke, D., Hagenbuch, B., Dong, Y., Malenka, R.C., Fritschy, J.M., Bluethmann, H., Feldon, J., Mohler, H. & Rudolph, U. (2005) A schizophrenia-related sensorimotor deficit links α3-containing $GABA_A$ receptors to a dopamine hyperfunction. *Proc. Natl. Acad. Sci. USA*, **102**, 17154-17159.

Yeo, M., Berglund, K., Augustine, G. & Liedtke, W. (2009) Novel repression of Kcc2 transcription by REST-RE-1 controls developmental switch in neuronal chloride. *J Neurosci*, **29**, 14652-14662.

Young, S., Taylor, M., Wu, S., Ikeda-Matsuo, Y., Kubera, C. & Bordey, A. (2012) NKCC1 knockdown decreases neuron production through $GABA_A$-regulated neural progenitor proliferation and delays dendrite development. *J Neurosci*, **32**, 13630-13638.

Zeller, A., Arras, M., Jurd, R. & Rudolph, U. (2007) Mapping the contribution of beta3-containing $GABA_A$ receptors to volatile and intravenous general anesthetic actions. *BMC Pharmacology*, **7**, 2.

Zeller, A., Crestani, F., Camenisch, I., Iwasato, T., Itohara, S., Fritschy, J. & Rudolph, U. (2008) Cortical glutamatergic neurons mediate the motor sedative action of diazepam. *Mol Pharmacol*, **73**, 282-291.

Zhu, P., Frank, T. & Friedrich, R. (2013) Equalization of odor representations by a network of electrically coupled inhibitory interneurons. *Nat Neurosci*, **16**, 1678-1686.

Table 1: Consequences of altered GABAergic function for neurodevelopmental and psychiatric disorders

Possible causes	Functional consequences	Functional deficit, disorder	Selected references
Reduced GABA synthesis, defective maturation of interneurons, altered excitatory-inhibitory balance	Abnormal opening/duration of critical windows of plasticity	Sensory, motor, or cognitive (e.g., language) deficits	(Hensch, 2005; Ehninger *et al.*, 2008; Bavelier *et al.*, 2010)
Mutations affecting extracellular matrix proteins, synapse formation and transcription factors; defective neurotrophin signaling	Anormal neuronal migration, interneuron differentiation, synapse formation (e.g., axon-initial segment)	Epilepsy, schizophrenia	(Lewis *et al.*, 2005; Galanopoulou, 2010; Heinrich *et al.*, 2011; Marín, 2012)
Mutations affecting genes involved in synaptogenesis (e.g., NLGNs), defective postsynaptic scaffold formation and intracellular signaling, impaired excitatory-inhibitory balance	Altered intracellular signaling, impaired dendrite development, spine maturation, reduced synaptic plasticity	Intellectual disabilities, Angelman syndrome, autism-spectrum disorders	(Südhof, 2008; Blundell *et al.*, 2009; Shen & Scheiffele, 2010; Pizzarelli & Cherubini, 2011)
Altered expression of $GABA_AR$ subunit genes, early-life stress, defective BDNF signaling	Abnormal $GABA_AR$ function at critical stages of brain development	Anxiety disorders, major depression	(Hong *et al.*, 2008; Maguire & Mody, 2009; Shen *et al.*, 2012; Smith, 2013; Vithlani *et al.*, 2013)

Figure legends

Figure 1

Differential distribution of $GABA_AR$ α subunit variants in the adult mouse forebrain. **A-E:** Each panel depicts in false colors, ranging from dark blue to red, orange, yellow, and white for maximal intensity, the relative staining intensity pattern of each subunit indicated, as determined by immunoperoxidase staining. α6 is not depicted, because it is not expressed in the forebrain. Note that each subunit has a unique distribution pattern, with partial overlap and complementarity to other α subunits. **F:** Main anatomical structures present in the images of panels A-E (ac, anterior commissure; cc, corpus callosum; AON, anterior olfactory nucleus; CA1, CA1 region of the hippocampus; CPu, caudate nucleus and putamen (= striatum); DG, dentate gyrus; GPe, globus pallidus, external; IC, inferior colliculus; nRT, thalamic reticular nucleus; OB, olfactory bulb; Pi, piriform cortex; Po, pontine nuclei; S1 primary somato sensory cortex; SC, superior colliculus; SNc, substantia nigra, pars reticulata; Su, subiculum; VP, ventral pallidum; VPL, ventral postero-lateral thalamic nucleus. Scale bar, 2 mm. Adapted from (Panzanelli *et al.*, 2011).

Figure 2

Distinction of postsynaptic and extrasynaptic $GABA_ARs$ detected by immunofluorescence staining and confocal laser scanning microscopy. The subcellular localization is based on the identification of postsynaptic sites positive for gephyrin and presynaptic GABAergic terminals positive for VGAT. **A:** α2 subunit staining in adult mouse CA1, revealing numerous brightly stained clusters around pyramidal cell bodies in the stratum pyramidale (sp) and on their dendrites in stratum oriens (so) and radiatum (sr). **A1-A4:** High magnification images taken from a section triple stained for α2 (red), gephyrin (yellow), and VGAT (blue) in stratum radiatum, depicting in single, double, and triple staining that α2 clusters are colocalized with gephyrin (A3; yellow) and apposed to VGAT-positive terminals (A2). **B:** α5 subunit staining in adult mouse CA1,depicting the granular appearance of the staining, with pyramidal cells in sp appearing as lightly stained structures with an unstained nucleus. **B1-B4:** at higher magnification, the granular staining lacks bright clusters (as seen for α2 in panel A1) and shows no obvious relationship to gephyrin clusters (B3; green) or VGAT-positive terminals (B4; blue). Scale bars: A-B, 20 μm; A1-A4, B1-B4, 5 μm. Adapted from (Panzanelli *et al.*, 2011).

Figure 3

Schematic depiction of major postsynaptic proteins interacting with $GABA_A$Rs and their putative organization in the PSD of a GABAergic synapse (see main text for details). **A:** Schematic depiction of key molecules of GABAergic synapses. **B:** Possible arrangement of gephyrin molecules, forming trimers as proposed from structural analysis (Sander *et al*., 2013), and models of scaffolding assembly, to which $GABA_A$Rs and collybistin bind. **C:** Basic molecular organization of the GABAergic PSD, depicting the presence of NL2 (interacting with presynaptic neurexin isoforms and with gephyrin), collybistin splice variants, in both active and inactive conformation, and interacting with the small GTPases CDC-42 and/or TC-10, in addition to gephyrin and $GABA_A$Rs. The exact roles of collybistin, and its enzymatic activity, remain hypothetical; the scheme shows a proposed function for gephyrin submembrane targeting, along with possible effects within the PSD itself to facilitate recruitment of $GABA_A$Rs moving via lateral diffusion in the membrane. The size of each molecule is depicted roughly relative to its molecular weight.

Graphical abstract

$GABA_A$ receptor heterogeneity arises through combinatorial assembly of a large family of subunits to generate multiple receptor subtypes. It is an important facet of the variety of GABAergic signaling in adult and developing CNS, and a key factor underlying GABAergic synaptic plasticity underlying excitatory/inhibitory balance in neuronal circuits. This review presents and discusses recent progress in elucidating the relevance of $GABA_A$ receptor heterogeneity for CNS function in health and disease.

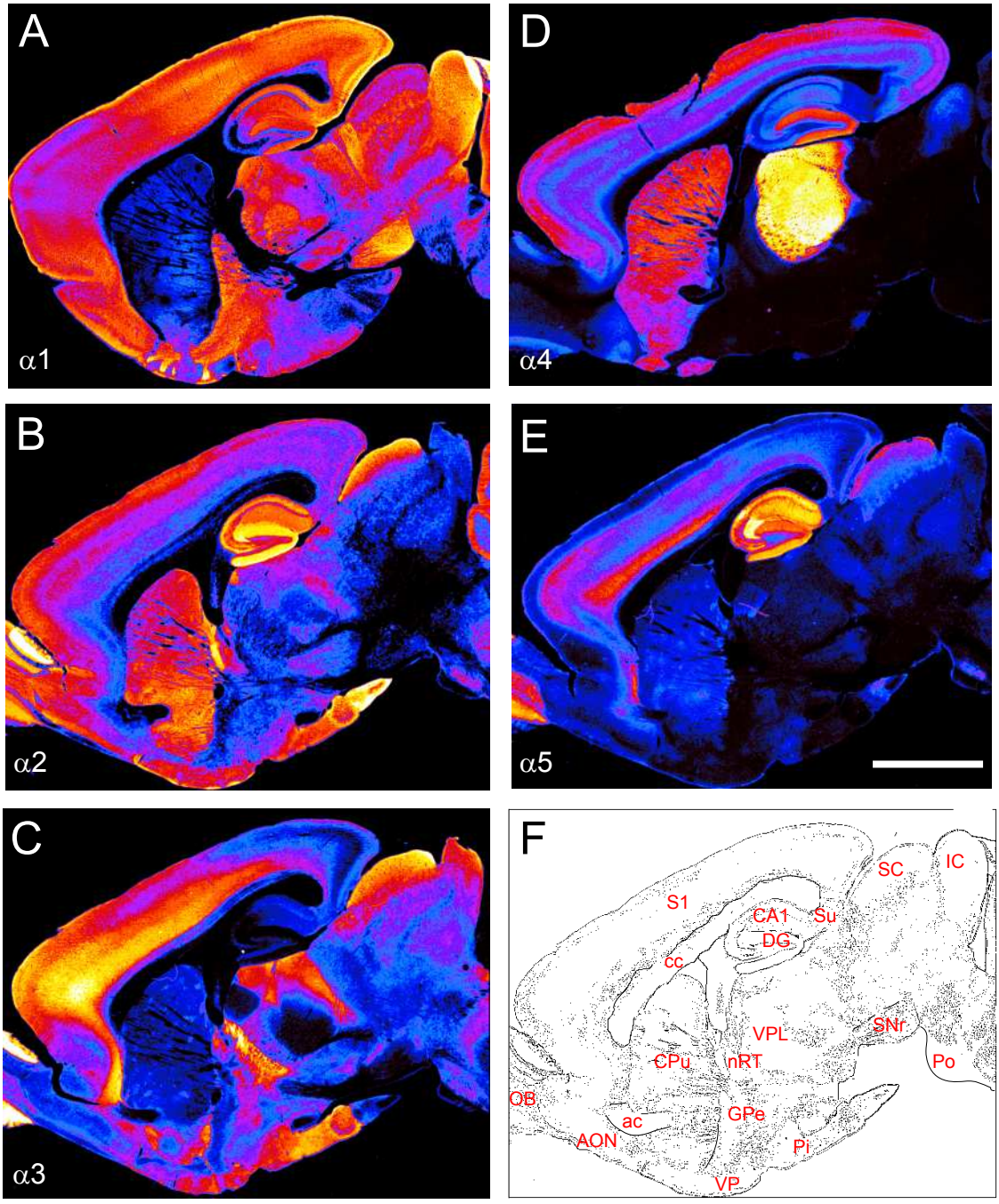
A
α1
D
α4
B
α2
E
α5
C
α3
F
S1
CA1
Su
DG
cc
SC
IC
VPL
SNr
CPu
nRT
Po
OB
ac
GPe
AON
Pi
VP

Figure 1

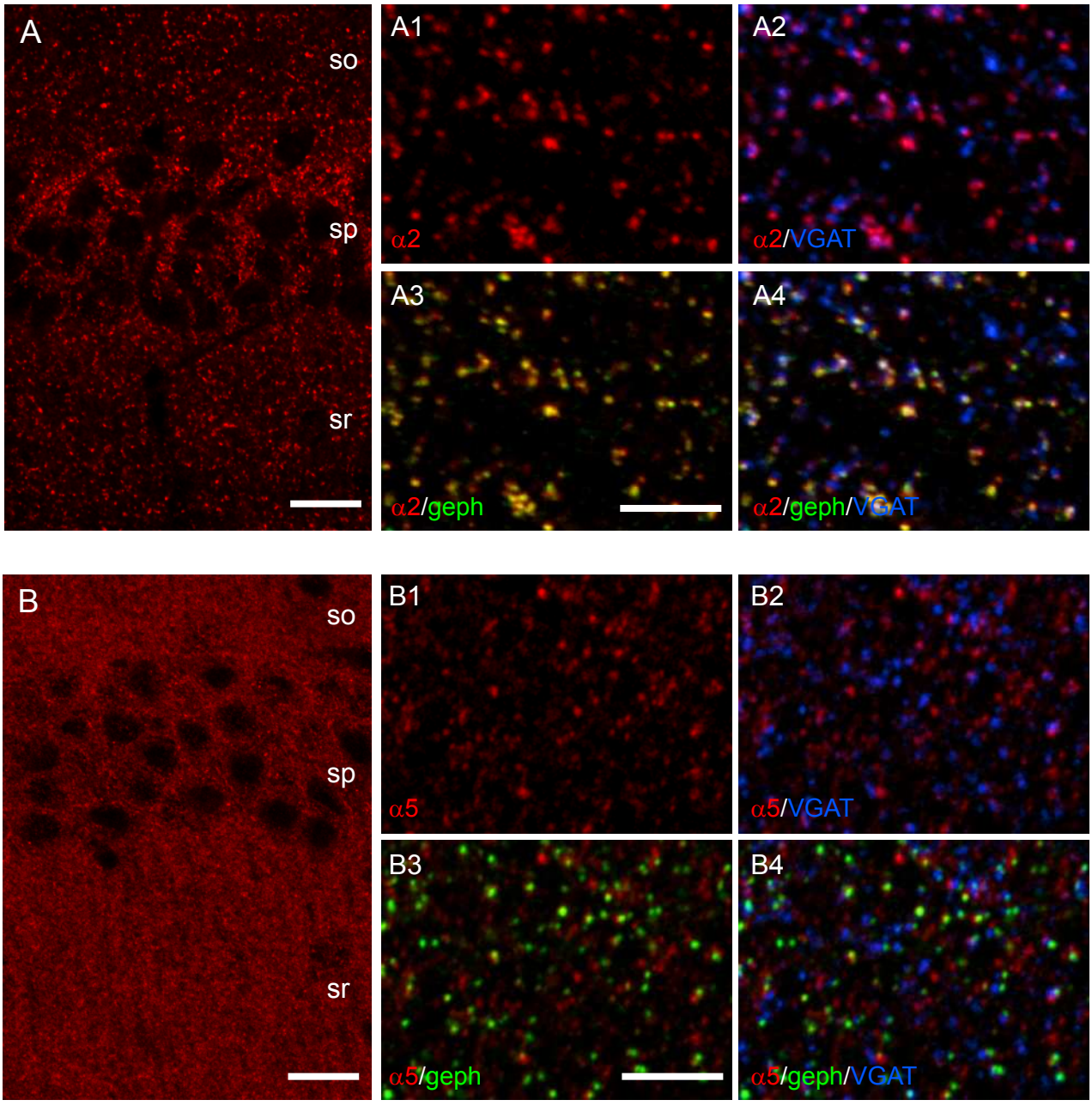
A
so
sp
sr
A1
α2
A2
α2/VGAT
A3
α2/geph
A4
α2/geph/VGAT
B
so
sp
sr
B1
α5
B2
α5/VGAT
B3
α5/geph
B4
α5/geph/VGAT

Figure 2

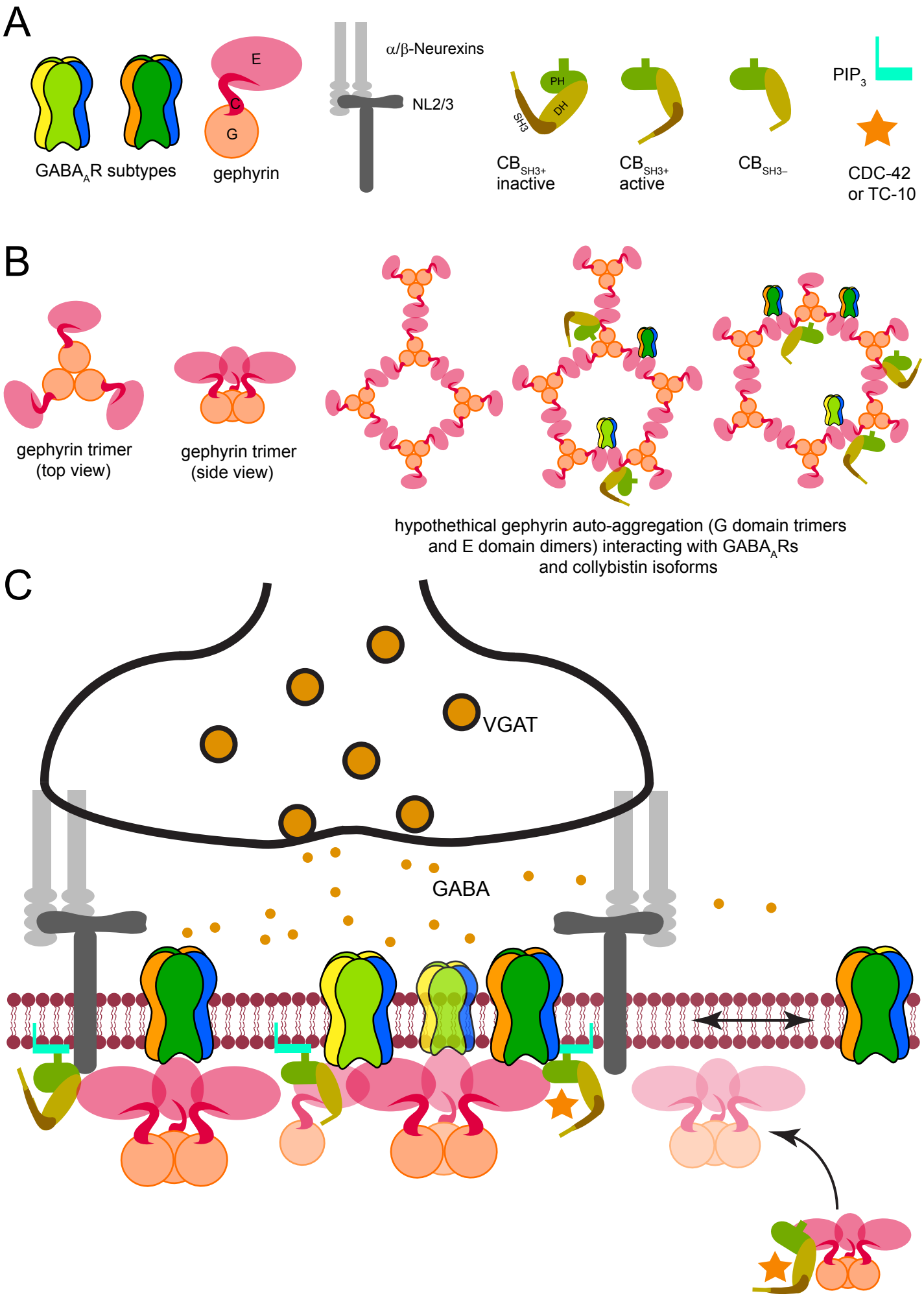
A
GABA$_A$R subtypes
E
C
G
gephyrin
α/β-Neurexins
NL2/3
PH
DH
SH3
CB$_{SH3+}$ inactive
CB$_{SH3+}$ active
CB$_{SH3-}$
PIP$_3$
CDC-42 or TC-10
B
gephyrin trimer (top view)
gephyrin trimer (side view)
hypothetical gephyrin auto-aggregation (G domain trimers and E domain dimers) interacting with GABA$_A$Rs and collybistin isoforms
C
VGAT
GABA

Figure 3